Pedpradab, S., Edrada, R.A., Ebel, R., Wray, V. & Proksch, P. New -carboline alkaloids from the Andaman sea sponge Dragmacidon sp. J. Nat. Prod. 67, 2113-2116

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JANUARY 2005

Impact Factor: 3.8 · DOI: 10.1021/np0401516 · Source: PubMed

New β-Carboline Alkaloids from the Andaman Sea Sponge *Dragmacidon* sp.

Suwigarn Pedpradab,[†,‡] RuAngelie Edrada,[‡] Rainer Ebel,[‡] Victor Wray,[§] and Peter Proksch*[,‡]

Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität, Universitätsstrasse 1, 40225 Düsseldorf, Germany, and Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 38124 Braunschweig, Germany

Received July 6, 2004

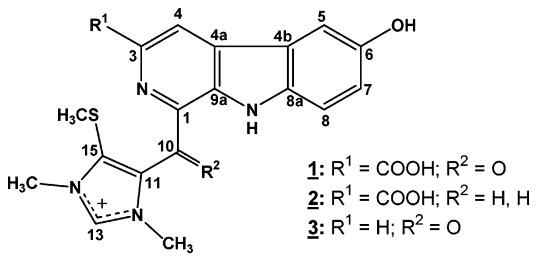
Chemical investigation of the hithertho undescribed sponge *Dragmacidon* sp. from the Andaman Sea afforded two new β-carboline alkaloids, which were named dragmacidonamines A (**1**) and B (**2**). The structures of the new compounds were unambiguously established on the basis of NMR spectroscopic (^{1}H, ^{13}C, COSY, ^{1}H detected direct, and long-range ^{13}C−^{1}H correlations) and mass spectrometric (EI and ESIMS) data. The 3-carboxylic-β-carboline moiety and the thiomethylated imidazolinium unit as found in dragmacidonamines are very rarely encountered in nature.

Alkaloids containing the β-carboline skeleton have been isolated from several marine invertebrates, which include hydroids[1] (*Aglaophenia*), bryozoans[2,3] (*Cribricellina*, *Catenicella*), soft corals[4] (*Lignopsis*), tunicates (*Eudistoma*,[5] *Didemnum*,[6] *Lissoclinum*,[7] *Ritterella*,[8] *Pseudodistoma*[9]), and various sponges. β-Carboline alkaloids are widely distributed among different orders of the class Demospongiae (e.g., Haplosclerida, Petrosida, Dictyocertida, Verongida, Halichondrida, and Homosclerophorida).[10] Alkaloids possessing the β-carboline moiety, as found in manzamines,[11] didemnolines,[12] eudistomins,[5a] fascaplysins,[13] plakortamines,[14] and shishijimicins[6c] to name a few, have been found to exhibit pronounced biological activity, such as antimicrobial, antitumor, antiviral, and insecticidal[15] activity. The hitherto undescribed sponge *Dragmacidon* sp., collected in the Andaman Sea, yielded two new β-carboline alkaloids, which we named dragmacidonamines A (**1**) and B (**2**). They are structurally related to hyrtiomanzamine, previously isolated from the New Caledonian sponge *Hyrtios erecta*. The latter compound was found to exhibit immunosuppressive activity in the B lymphocytes reaction assay.[16]

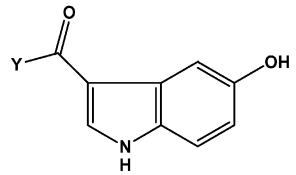
Dragmacidon sp. belongs to the family Axinellidae, order Halichondrida.[17] A literature survey[10] showed that only seven papers on sponges of the genus *Dragmacidon* had been published, and these sponges were reported to yield cytotoxic bis(indole) piperazine alkaloid derivatives.[18] In this study, β-carboline alkaloids were isolated for the first time from the sponge genus *Dragmacidon*.

The undescribed sponge *Dragmacidon* sp. was collected via scuba at a depth of 40 feet and subjected to extraction and chromatographic isolation of its secondary metabolites. The β-carboline congeners **1** and **2** as well as three known indole alkaloids, **4**, **5**, and **6**, were isolated from the methanol-soluble extract through a reversed-phase column followed by gel size exclusion chromatography on a Sephadex LH-20. Dragmacidonamines A (**1**) and B (**2**), which were present as the major metabolites, were separated from the aforementioned minor components by reversed-phase semipreparative HPLC.

The structures of the known compounds formyl-5-hydroxyindole[19] (**4**), 5-hydroxy-3-(hydroxyacetyl)-1*H*-indole,



1: R^1 = COOH; R^2 = O
2: R^1 = COOH; R^2 = H, H
3: R^1 = H; R^2 = O



4: Y = H
5: Y = CH_2OH
6: Y = OH

also known as hyrtiosin A[19] (**5**), and 5-hydroxy-1H-indole-3-carboxylic acid[20] (**6**) were identified by comparison of their ^{1}H and ^{13}C NMR data with those found in the literature.[19] Alkaloids **4** and **5** were previously isolated from the sponge *Hyrtios erecta*.

The structures of the new compounds were identified unambiguously by 1D and 2D NMR spectroscopy. Through-bond homonuclear (^{1}H−^{1}H COSY) and heteronuclear (long-range ^{13}C−^{1}H) correlations were used to establish assignments and atom connectivities. Chemical shifts were compared with literature data for compounds containing similar structural subunits.

Dragmacidonamine A (**1**) was obtained as an orange oil and showed a molecular ion peak at *m/z* 397 $[M]^+$ in both the EIMS and positive ESIMS experiments. The $[M + H]^+$ pseudomolecular ion peak was not observable in the positive mode in ESI. This suggested the occurrence of a protonated nitrogen function in the compound which gave the molecule a positive net charge and consequently exhibited a molecular ion peak identical to that obtained through EI. The observed molecular ion peak was consistent with that found in the negative ESIMS spectrum of compound **1**, which showed a pseudo-molecular ion peak at *m/z* 395.8 $[M - H]^-$. Both the (+)ESIMS/MS and EI spectra also gave a significant base peak ion at *m/z* 353 $[M - CO_2]^+$, which was attributable to the presence of a carboxylic function in the molecule. The HREIMS corresponded to the molecular formula $C_{19}H_{17}N_4O_4S.^+$ The ^{1}H and ^{13}C NMR spectra (Table 1) were comparable to those of hyrtiomanzamine (**3**).[16] Compound **1** had a 44 mass unit difference from hyrtiomanzamine (**3**). As found in **3**, the ^{1}H NMR spectrum of **1** revealed the occurrence of an ABX

* To whom correspondence should be addressed. Tel: 0049/211-8114163. Fax: 0049/211-8111923. E-mail: proksch@uni-duesseldorf.de.
† Permanent address: Department of Marine Sciences, Faculty of Sciences and Fisheries Technology, Rajamangala Institute of Technology, Trang Province, Thailand.
‡ Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität.
§ Gesellschaft für Biotechnologische Forschung.

10.1021/np0401516 CCC: $27.50
Published on Web 12/01/2004

Table 1. NMR Data for Compounds **1** and **2** in DMSO-d_6 (δ in ppm)

	1			**2**		
	δ_C	δ_H (#H, m)	HMBC (δ_H to δ_C)	δ_C	δ_H (#H, m)	HMBC (δ_H to δ_C)
1	134.1 (s)			142.6 (s)		
3	132.3 (s)			135.7 (s)		
4	122.3 (d)	9.15 (brs)	C-1, C-3, C-4b, C-9a, COOH	119.0 (d)	8.69 (brs)	C-4a, C-4b, C-9a, COOH
4a	132.1 (s)			127.5 (s)		
4b	121.1 (s)			122.2 (s)		
5	106.5 (d)	7.78 (brd, J = 1.9 Hz)	C-4a, C-6, C-7, C-8a	106.0 (d)	7.61 (brd, J = 2.2 Hz)	C-4a, C-6, C-7, C-8a
6	152.6 (s)			151.9 (s)		
7	119.0 (d)	7.20 (dd, J = 1.9, 8.2 Hz)	C-5, C-6, C-8a	119.1 (d)	7.10 (dd, J = 2.2, 9.5 Hz)	C-5, C-8a
8	114.0 (d)	7.69 (d, J = 8.2 Hz)	C-4b, C-6, C-8a	116.0 (d)	7.69 (d, J = 9.5 Hz)	C-4b, C-6
8a	136.2 (s)			135.1 (s)		
9a	133.4 (s)			135.7 (s)		
10	183.9 (s)			28.0 (t)	4.80 (s)	C-1, C-9a, C-11, C-13, C-15
11	134.8 (s)			125.7 (s)		
13	140.4 (d)	9.55 (s)	C-11, C-15, NCH_3-12, NCH_3-14	138.1 (d)	9.30 (s)	C-11, C-15, NCH_3-12
15	131.9 (s)			137.3 (s)		
COOH	166.2 (s)			166.8 (s)		
NCH_3	34.2 (q)	4.10 (s)	C-11, C-13	35.0 (q)	3.87 (s)	C-11, C-13
NCH_3	37.8 (q)	3.91 (s)		36.0 (q)	3.97 (s)	C-13, C-15
SCH_3	18.6 (q)	2.50 (s)	C-15	18.1 (q)	2.24 (s)	C-15
NH		12.40 (brs)	C-1, C-4a, C-4b		12.40 (brs)	

spin system which exemplified a 1,3,4-trisubstituted benzene, as demonstrated by doublet signals at 7.78 (br d, J = 1.9 Hz), 7.20 (dd, J = 1.9, 8.2 Hz), and 7.69 ppm (d, J = 8.2 Hz). The β-carboline unit was established through the observed long-range HMBC correlations of the broad N*H* singlet at δ_H 12.4 with carbons at δ_C 121.1 (C-4b), 132.1 (C-4a), and 134.1 ppm (C-1). However, *ortho* protons on the pyridine ring were not discernible as observed in **3**. The substitution pattern on the pyridine part of the compound was verified through the HMBC correlations of the methine singlet at δ_H 9.15 with the quaternary carbons at δ_C 121.1 (C-4b) and 133.4 ppm (C-9a), which suggested the C-4 position of the methine singlet. This implied the occurrence of a 1,3,6-trisubstituted-β-carboline moiety in **1**. Furthermore, the presence of a carboxylic acid substituent at C-3 was proved by the HMBC cross-peak of H-4 with a carbonyl resonance at δ_C 166.2, primarily not detectable from its ^{13}C NMR spectrum due to the broadness of the signal. The ^{13}C NMR spectrum of **1** revealed only one carbonyl signal at δ_C 183.9, which corresponded to the keto unit at C-10 as found in **3**. The ^{13}C NMR data for the pyridine ring were comparable to those of the 2-methyl-9H-pyrido[3,4*b*]-indole-3-carboxylic acid, a β-carboline isolated from the soft coral *Lignopsis spongiosum*.[4] In addition, the proton chemical shift of H-4 at δ_H 9.15 was also compatible with that observed in related compounds, as in the latter alkaloid (δ_H 9.21, in DMSO-TFA)[4] and in 1-acetyl-3-carboxymethoxy-β-carboline (δ_H 9.05, in $CDCl_3$), which was isolated from a Chilean Solanaceae, *Vestia lycioides*.[21]

The proton and carbon chemical shifts of the methyl signals were very similar to those found in the imidazolium nucleus of hyrtiomanzamine.[16] *N*-methyl singlets at δ_H 4.10 and 3.91 ppm were detectable with their corresponding carbon resonances at δ_C 34.2 and 37.8 ppm, respectively. The ^1H NMR data of the *N*-methyl functions were also closely related to those in norzooanemonin (δ_H 3.97 and 3.87, in D_2O), an alkaloid isolated from the Carribean gorgonian *Pseudopterogorgia americana*.[22] Observed resonances at δ_H 2.50 and δ_C 18.6 ppm were assigned to a thiomethyl group. The presence of the thiomethyl function was deduced from the carbon−proton coupling constant value of 140.0 Hz as detected in hyrtiomanzamine ($^1J_{C-H}$ = 141.7 Hz),[16] didemnoline A ($^1J_{C-H}$ = 142.0 Hz),[12] and varamin B ($^1J_{C-H}$ = 142.0 Hz).[23] The *N*,*N*-dimethyl imidazole ring system has been verified from the long-range HMBC correlations of the proton at δ_H 9.55 (H-13) with the *N*-methyl carbons at δ_C 34.2 and 37.8 as well as with the quaternary carbons at δ_C 134.8 (C-11) and 131.9 (C-15). The imidazolium proton H-13 showed a direct HMBC correlation with the carbon resonating at δ_C 140.4, which was also comparable to that found in **3**. Dragmacidonamine A was unambiguously elucidated to be the 3-carboxylic acid derivative of hyrtiomanzamine.

Dragmacidonamine B (**2**) was obtained as a slightly brown-colored oil, which showed a molecular ion peak at *m/z* 383 $[M]^+$ in both the EIMS and positive ESIMS experiments, while its negative ESIMS spectrum showed a pseudo-molecular ion peak at *m/z* 382.2 $[M - H]^-$. The molecular formula $C_{19}H_{19}N_4O_3S^+$ was established by HRE-IMS. Compound **2** had a 14 mass unit difference from dragmacidonamine A (**1**). Both the (+)ESIMS/MS and EI spectra gave a significant base peak ion at *m/z* 339 $[M - CO_2]^+$, which also ascribed the occurrence of a carboxylic unit in the molecule as in compound **1**. Additional evidence was the observable (−)ESIMS/MS fragment ion at *m/z* 338.4 $[(M - CO_2) - H]^-$. The ^1H and ^{13}C NMR spectra (Table 1) were comparable to those of compound **1**. The ^1H NMR data of **2** indicated the presence of a similar 1,3,6-trisubstituted-β-carboline moiety and a thiomethylated *N*,*N*-dimethyl imidazole ring system identical to that found in the latter derivative (**1**). However, the proton resonances for compound **2** were rather shielded when compared to those in **1**. Notable differences in the ^{13}C NMR chemical shifts between the two compounds were also discernible, and the most significant were observed for C-1, which was deshielded to δ_C 142.6 (Δ 8.5 ppm), while C-11 was shielded to δ_C 127.5 (Δ 9.1 ppm). From the DEPT spectrum of **2**, the appearance of a methylene signal was detected at δ_C 28.0, which then exhibited a direct correlation with a methylene singlet at δ_H 4.80, as revealed by its HMBC spectrum. Furthermore, the methylene singlet at δ_H 4.80 showed long-range HMBC correlations with carbon signals belonging to both the β-carboline unit (C-1 and C-9a) and the imidazole moiety (C-11, C-13, C-15). In addition, the absence of a carbonyl signal at δ_C 183.9 as previously found in compound **1** implied the disappearance of the keto function at C-10, which consequently accounted for the 14 mass unit difference of congener **2** from compound **1**. This

likewise explained the deshielding and shielding effect on C-1 and C-11, respectively, to which both moieties were connected together through a sp^3 bridge. The ^1H and ^{13}C NMR data of the β-carboline unit, particularly the pyridine portion of dragmacidonamine B (**2**), were compatible with that of cordifoline and desoxycordifoline ($\delta_{H-4/C-4}$ 8.69/114.2, δ_{C-1} 142.9, $\delta_{C-3/C-9a}$ 135.6, and δ_{C-4a} 128.4).[24] Cordifolines are β-carboline 3-carboxylate glucoalkaloids isolated from the heartwood of *Adina*[24b] and *Nauclea*[24c] species, both from the family Naucleaceae and also from an endemic East African species, *Strychnos mellodora*.[24a] From these data, it was concluded that compound **2** is the deoxygenated congener of dragmacidonamine A and was named dragmacidonamine B.

3-Carboxylate-β-carbolines are rarely found in nature, and a recent literature survey of isolated natural products showed only 20 compounds that contain this function. Most of these compounds, known as cordifolines glucoalkaloids, occur in several plant species of the family Naucleacea, which includes the genera *Adina*[24b] and *Nauclea*.[24c] Esterified congeners occur in the Chinese medicinal plant *Picrasma quassinoides* (Simaroubaceae),[25] while 1-furanyl derivatives, known as flazines, were found in the seeds of *Brucea javanica*[26] and the pressed juice of the blackcurrant (*Ribes nigrum*, Fam. Grossulariaceae).[27] Aerial parts of *Vestia lycioides* (Solanaceae)[21] and the bark of several species of *Aspidosperma* (Apocynaceae)[28] yielded 1-acetyl and 1-methyl congeners, respectively. Ester forms of these derivatives have been reported as active benzodiazepine tranquilizer antagonists. From microbial sources, a 1-quinolinyl derivative named lavendamycin and a 1-ethenyl congener were isolated from *Streptomyces lavendulae*[29] and *Nocardiopsis dassovillei*,[30] respectively. Lavendamycin has been reported as an antineoplastic antibiotic and a potent HIV-reverse transcriptase inhibitor,[29a] while 1-ethenyl-3-carboxylate-β-carboline has been patented as an antiinflammatory and antitumor agent. In the present case only dragmacidonamine A showed moderate cytotoxic activity toward the murine leukemia cell line L5178Y.

To date, the quaternary alkaloid 2-methyl-3-carboxylate-β-carboline from the coelenterate *Lignopsis spongiosum*[4] is the sole congener that has been isolated from a marine environment. The dragmacidonamines are hithertho the first 3-carboxylate-β-carbolines isolated from the phylum Porifera.

Experimental Section

General Experimental Procedures. ^1H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded at 300 K on Bruker DPX, ARX 300, or AVANCE DMX 600 NMR spectrometers. Mass spectra were recorded on a ThermoFinnigan LCQ Deca for ESIMS and ESIMS/MS measurements, and EIMS was measured on a Finnigan MAT TSQ-7000 mass spectrometer, respectively, while HREIMS spectra were obtained on a Finningan MAT 95 mass spectrometer. For HPLC analysis, samples were injected into an HPLC system coupled to a photodiode-array detector (Dionex, Munich, Germany). Routine detection was at 254 nm in aqueous MeOH. The separation column (125 × 4 mm, i.d.) was prefilled with C_{18} (Knauer, Berlin, Germany). Semipreparative HPLC was performed on a Merck-Hitachi Eurosher-100, pump L-7100, and L-7400 UV detector. The separation column (8 × 250 mm) was prepacked with Eurosphere C_{18} (Knauer, Berlin, Germany). The compounds were eluted with mixtures of MeOH and H_2O at a flow rate of 5 mL/min.

Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurement. TLC was performed on TLC plates precoated with Si 60 F_{254} (Merck, Darmstadt, Germany). The compounds were detected from their UV absorbance at 254 nm and by spraying the TLC plates with Dragendorff reagent.

Animal Material. The sponge was taxonomically identified as *Dragmacidon* sp. and belongs to the class Demospongiae, order Axinellida, family Axinellidae. The sponge was massive, yellow in color with a rough texture. The sponge samples were collected in April 2000 by scuba diving at a depth of 40 feet in the Andaman Sea, near the coast of Trang Province in Thailand. Voucher specimens have been deposited at the Faculty of Sciences and Fisheries Technology, Rajamangala Institute of Technology, Trang Province, Thailand, and in the Zoological Museum Amsterdam under the registration number ZMA POR 16782.

Extraction and Isolation. Samples (500 g, wet weight) were frozen immediately and freeze-dried prior to extraction. The sponge *Dragmacidon* sp. was macerated in MeOH. The total alcoholic extract was concentrated and dried in vacuo. The dried extract was then reconstituted in aqueous MeOH and shaken with EtOAc, which gave 2.34 g of dried organic extract. The EtOAc extract was chromatographed on a C_{18} reversed-phase column and eluted with MeOH/H_2O (6:4), from which eight fractions were obtained. Dragmacidonamine B (**2**, 10 mg) was obtained from the fourth fraction. The third fraction was subjected to gel size exclusion chromatography on a Sephadex LH-20 column using MeOH as eluent to yield dragmacidonamine A (**1**, 8 mg) and the known indole alkaloids **4** (5 mg), **5** (3 mg), and **6** (4 mg). The isolated compounds were further purified by semipreparative reversed-phase HPLC utilizing a 30 min gradient program of 10% to 50% MeOH with 0.1% TFA in H_2O.

Dragmacidonamine A (1): yellow amorphous solid; UV λ_{max} (MeOH) 221, 254, 317 nm; ^1H and ^{13}C NMR data, see Table 1; (+)ESIMS *m/z* 397.2 $[M]^+$; (+)ESIMS/MS *m/z* 353.2 $[M - CO_2]^+$; (−)ESIMS *m/z* 395.8 $[M - H]^-$, 441.5 $[(M + HCOOH) - H]^-$; EIMS *m/z* 397 $[M]^+$ (60), 353 $[M - CO_2]^+$ (100), 306 (20); HRESIMS 397.0970 (397.0971 calcd for $C_{19}H_{17}N_4O_4S^+$).

Dragmacidonamine B (2): slightly brown oil; UV λ_{max} (MeOH) 222, 242, 282 nm; ^1H and ^{13}C NMR data, see Table 1; (+)ESIMS *m/z* 383.4 $[M]^+$; (+)ESIMS/MS *m/z* 339.4 $[M - CO_2]^+$; (−)ESIMS *m/z* 382.1 $[M - H]^-$; (+)ESIMS/MS *m/z* 338.4 $[(M - CO_2) - H]^-$; EIMS *m/z* 383 $[M]^+$ (80), 339 $[M - CO_2]^+$ (100), 293 (40); HRESIMS 383.1180 (383.1178 calcd for $C_{19}H_{19}N_4O_3S^+$).

Cytotoxicity Assay. Antiproliferative activity was examined against several cell lines and was determined through an MTT assay as described earlier.[31]

Acknowledgment. This study was supported by a grant of the BMBF awarded to P.P. A scholarship granted and financed by the Rajamangala Institute of Technology (Ministry of Higher Education) to S.P. is gratefully acknowledged. We thank C. Kakoschke and B. Jaschok-Kentner for recording NMR data (GBF, Braunschweig). The authors are grateful to Dr. R. Van Soest for the taxonomical identification of the sponge, Dr. M. Nimtz, GBF, Braunschweig, for the HREIMS measurements, and Prof. W. E. G. Müller, Institute für Physiologische Chemie, Univerität Mainz, Germany, for the cytotoxicity assay.

References and Notes

(1) Aiello, A.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* **1987**, *43*, 5929−5932.
(2) (a) Prinsep, M. R.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod*. **1991**, *54*, 1068−1076. (b) Harwood: D. T.; Urban, S.; Blunt, J. W.; Munro, M. H. G. *Nat. Prod. Res*. **2003**, *17*, 15−19.
(3) Beutler, J. A.; Cardellina, J. H.; Prather, T.; Shoemaker, R. H.; Boyd, M. R.; Snader, K. M. *J. Nat. Prod*. **1993**, *56*, 1825−1826.
(4) Cabrera, G. M.; Seldes, A. M. *J. Nat. Prod*. **1999**, *62*, 759−760.
(5) (a) Schupp, P.; Poehner, T.; Edrada, R.; Ebel, R.; Berg, A.; Wray, V.; Proksch, P, *J. Nat. Prod*. **2003**, *66*, 272−275, and references therein. (b) Schupp, P.; Proksch, P.; Wray, V. *J. Nat. Prod.* **2002**, *65*, 295−298, and references therein. (c) Rashid, M. A.; Gustafson, K. R.; Boyd, M. R. *J. Nat. Prod*. **2001**, *64*, 1454−1456.
(6) (a) Kearns, P. S.; Coll, J. C.; Rideout, J. A. *J. Nat. Prod.* **1995**, *58*, 1075−1076. (b) Foderaro, T. A.; Barrows, L. R.; Lassota, P.; Ireland, C. M. *J. Org. Chem.* **1997**, *62*, 6064−6065. (c) Oku, N.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc.* **2003**, *125*, 2044−2045.

(7) (a) Searle, P. A.; Molinski, T. F. *J. Org. Chem*. **1994**, *59*, 6600−6605. (b) Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Francisco, C. *J. Nat. Prod.* **1994**, *57*, 528−533.
(8) (a) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem*. **1989**, *42*, 1201−1206. (b) Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Lett.* **1988**, *29*, 2255−2256.
(9) (a) Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **1998**, *61*, 959−960. (b) Chbani, M.; Pais, M.; Delauneux, J. M.; Debitus, C. *J. Nat. Prod.* **1993**, *56*, 99−104.
(10) MarinLit. 2003. A marine literature database produced and maintained by the Department of Chemistry, University of Canterbury.
(11) (a) Hu, J.-Feng; Hamann, M. T.; Hill, R.; Kelly, M. In *The Alkaloids*. *Chemistry and Biology*; Cordell, G. A., Ed.; Elsevier-Academic: San Diego, CA, 2003; Vol. 60, pp 207−285, and references therein. (b) El Sayed, K. A.; Kelly, M.; Kara, U. A. K.; Ang, K. K. H.; Katsuyama, I.; Dunber, D. C.; Khan, A. A.; Hamann, M. T. *J. Am. Chem. Soc.* **2001**, *123*, 1804−1808, and references therein.
(12) Schumacher, R. W.; Davidson, B. S. *Tetrahedron* **1995**, *51*, 10125−10130.
(13) (a) Segraves, N. L.; Robinson, S. J.; Garcia, D.; Said, S. A.; Fu, X.; Schmitz, F. J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2004**, *67*, 783−792, and references therein. (b) Segraves, N. L.; Lopez, S.; Johnson, T. A.; Said, S. A.; Fu, X.; Schmitz, F. J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P. *Tetrahedron Lett*. **2003**, *44*, 3471−3475, and references therein. (c) Charan, R. D.; McKee, T. C.; Boyd, M. R. *Nat. Prod. Res.* **2004**, *18*, 225−229.
(14) Sandler, J. S.; Colin, P. L.; Hooper, J. N. A.; Faulkner, D. J. *J. Nat. Prod.* **2002**, *65*, 1258−1261.
(15) (a) Peng, J.; Shen, X.; El Sayed, K. A.; Dunbar, D. C.; Perry, T. L.; Wilkins, S. P.; Hamann, M. T.; Bobzin, S.; Huesing, J.; Camp, R.; Prinsen, M.; Krupa, D.; Wideman, M. A. *J. Agric. Food Chem.* **2003**, *51*, 2246−2252. (b) Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Muller, W. E. G.; Van Soest, R. *J. Nat. Prod.* **1996**, *59*, 1056−1060.
(16) Bourguet-Kondracki, M. L.; Martin, M. T.; Guyot, M. *Tetrahedron Lett*. **1996**, *37*, 3457−3460.
(17) van Soest, R. W. M.; Hooper, J. N. A. Order Halichondrida. In *Systema Porifera: A Guide to the Classification of Sponges*, 1st ed.; van Soest, R. W. M., Hooper, J. N. A., Eds.; Kluwer Academic/Plenum Publishers: New York, 2002; Vol. 1, pp 721−787.
(18) (a) Kohmoto, S.; Kashman, Y.; McConnell, O. J.; Rinehart, K. L.; Wright, A.; Koehn, F. J. *Org. Chem*. **1988**, *53*, 3116−3118 (b) Mancini, I.; Guella, G.; Debitus, C.; Waikedre, J.; Pietra, F. *Helv. Chim. Acta* **1996**, *79,* 2075−2082. (c) Cutignano, A.; Bifulco, G.; Bruno, I.; Casapullo, A.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron* **2000**, *56*, 3743−3748.
(19) Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699−7702.
(20) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*; Aldrich Chemical Co.: Milwaukee, WI, 1993; Vol. 3, p 139: C.
(21) Faini, F.; Castillo, M.; Torres, R. *Phytochemistry* **1978**, *17*, 338.
(22) Weinheimer, A. J.; Metzner, E. K.; Mole, M. L., Jr. *Tetrahedron* **1973**, *29*, 3135−3136.
(23) Molinski, T. F.; Ireland, C. M. *J. Org. Chem*. **1989**, *54*, 4256−4259.
(24) (a) Brandt, V.; Tits, M.; Geerlings, A.; Frederich, M.; Penelle, J.; Delaude, C.; Verpoorte, R.; Angenot, L. *Phytochemistry* **1999**, *51*, 1171−1176. (b) Balazs, B.; Toth, G.; Rao, P. S.; Narender, T.; Rao, N. Subba; Horvath, Gy.; Duddeck, H. *Magn. Reson. Chem*. **1999**, *37*, 751−753, and references therein. (c) Adeoye, A. O.; Waigh, R. D. *Phytochemistry* **1983**, *22*, 2097−2098.
(25) Koike, K.; Ohmoto, T.; Ikeda, K. *Phytochemistry* **1990**, *29*, 3060−3061.
(26) Su, B.-N.; Chang, L. C.; Park, E. J.; Cuendet, M.; Santarsiero, B. D.; Mesecar, A. D.; Mehta, R. G.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *Planta Med.* **2002**, *68*, 730−733.
(27) Blech, St.; Budzikiewicz, H.; Dill, D. *Z. Naturforsch., C: Biosci.* **1994**, *49*, 540−544.
(28) Medina, J. D.; Di Genova, L. *Planta Med.* **1979**, *37*, 165−167.
(29) (a) Behforouz, M.; Cai, W.; Stocksdale, M. G.; Lucas, J. S.; Jung, J. Y.; Briere, D.; Wang, A.; Katen, K. S.; Behforouz, N. C. *J. Med. Chem*. **2003**, *46*, 5773−5780. (b) Balitz, D. M.; Bush, J. A.; Bradner, W. T.; Doyle, T. W.; O'Herron, F. A.; Nettleton, D. E. *J. Antibiot.* **1982**, *35*, 259−265.
(30) Otsuka Pharmaceutical Co., Ltd., Japan. Jpn. Kokai Tokkyo Koho. 1982, 14 pp. Patent: JP 81-56155 19810413. CAN 98:126057 AN 1983:126057.
(31) (a) Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Müller, W. E. G.; Van Soest, R. W. M. *J. Nat. Prod.* **1996**, *59*, 1056−1060. (b) Kreuter, M. H.; Robitzki, A.; Chang, S.; Steffen, R.; Michaelis, M.; Kljajic, Z.; Bachmann, M.; Schröder, H. C.; Müller, W. E. G. *Comp. Biochem. Physiol.* **1992**, *101C*, 183−187.

NP0401516