Purealidin S and Purpuramine J, Bromotyrosine Alkaloids from the Fijian Marine Sponge *Druinella* sp.

Jioji N. Tabudravu and Marcel Jaspars*

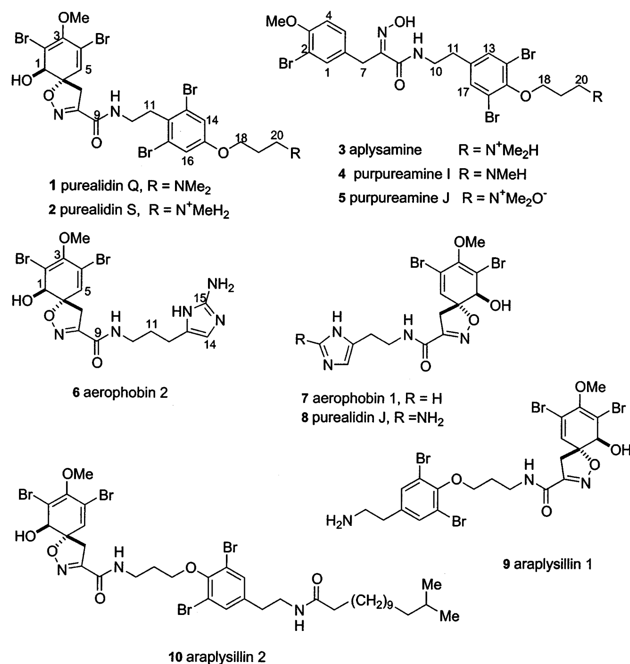
Marine Natural Products Laboratory, Department of Chemistry, University of Aberdeen, AB24 3UE, Scotland, U.K.

Received June 14, 2002

Two bromotyrosine alkaloids, purealidin S (**2**) and purpuramine J (**5**), were isolated from the Fijian marine sponge *Druinella* sp. Eight known bromotyrosine compounds were also isolated. This is the first report of a bromotyrosine N-oxide containing alkaloid. These two compounds were found to have moderate cytotoxic activity. In addition, bioassay data for the eight known bromotyrosine metabolites are reported.

Introduction

Sponges of the order Verongida have been known to produce a wide range of bromotyrosine compounds with interesting biological activities. The unusually large number of biosynthetically related compounds has been linked to the potentially large number of chemical variations that are possible within the aromatic ring and/or side chains of the tyrosine moiety. The aromatic ring can be either maintained, reduced, or oxidized[1] or mono- or dibrominated.[2,3] The bromotyrosine moiety can also undergo rearrangement to a spirooxepinisoxaline system, presumably via a common oxide intermediate as in the cases of the psammaplysins.[4−6] Alternatively, the bromotyrosine can rearrange to form the spirocyclohexadienylisoxazole system.[6] The bromotyrosine units can also link up to form linear chains through amide bonds as in the case of fistularin-3[7] or through ether bonds as in the formation of macrocyclic bastadins.[8−10] Variations in side chains include incorporation of cysteine,[11,12] homoserine,[13] and histidine[14] as their decarboxylated amines. Incorporation of pyridine[15] and pyrizinone[16] moieties has also been found. Bromotyrosine-derived compounds have been observed to show a wide range of interesting biological activities including antiviral,[17,18] antibiotic,[1,3,7,18−21] Na^+/K^+ ATPase inhibitory,[15,22−25] anti-HIV,[5,26] antifouling,[27−30] and histamine-H_3 antagonist.[31] Furthermore, they have been observed to show interesting anticancer activities.[6,8,16,18,32]



Results and Discussion

A freeze-dried sample of *Druinella* sp. was extracted and subjected to solvent partitioning. Interesting ^1H and ^{13}C NMR resonances were detected in the CH_2Cl_2, butanol, and MeOH fractions. The CH_2Cl_2 fraction was subjected to size exclusion chromatography followed by normal-phase HPLC to yield compounds **1**−**5**. The MeOH fraction yielded compounds **6**−**8**, while the butanol fraction yielded compounds **9** and **10**.

Inspection of the ^1H and ^{13}C NMR, DEPT-135, and MS indicated that eight compounds were known: purealidin Q (**1**),[33] aplysamine 2 (**3**),[34] purpuramine I (**4**)[3], aerophobin 2 (**6**),[14] aerophobin 1 (**7**),[14] purealidin J (**8**),[35,36] araplysillin 1 (**9**), and araplysillin 2 (**10**).[22]

The ^{13}C NMR spectrum of purealidin S (**2**) indicated the presence of 22 carbons (Table 1), one O-methyl (δ_C 60.1), one ammonium-methyl group (δ_C 32.0), six methylenes (δ_C 71.4, 48.8, 40.9, 39.0, 34.7, 27.9), three sp^2 methines (δ_C 134.1, 134.1, 132.0), one sp^3 methine (δ_C 75.0), eight sp^2 (δ_C 154.8, 151.9, 148.9, 139.8, 122.5, 118.4, 118.4, 113.8) and one sp^3 (δ_C 92.0) quaternary carbon, and an amide carbonyl (δ_C 161.2). These accounted for all 22 carbons. All protonated carbons were assigned by an HSQC experiment.[37] The low-resolution mass spectrum displayed an isotopic cluster (726.85, 728.85, 730.85, 732.86, 734.85), consistent with the presence of four bromine atoms. The high-resolution mass spectrum (HREIMS) gave an *m*/*z* of 728.8676 $[M + H]^+$ for a Δ 0.8 mmu calculated for $C_{22}H_{27}N_3O_5{}^{79}Br_3{}^{81}Br$. An unsaturation number of 10 was calculated, which in conjunction with five aromatic double bonds, one carbonyl, and one amide carbonyl suggested the presence of three rings in the molecule.

Use of one- and two-dimensional NMR data (Table 1) enabled the construction of four substructures (Figure 1). Inspection of ^1H, ^{13}C, and $^1H-^1H$ COSY NMR spectra suggested that the following proton signals belonged to the same spin system: δ_H 6.36 (sp^2 methine), δ_H 4.08 (sp^3 methine), 3.71 (OMe), and an AB system (δ_H 3.73 and δ_H 3.01 each 1H) characteristic of a spirocyclooxazoline ring system previously encountered in other Verongida compounds.[38] HMBC correlations from C-1 to H-5, H-7A/B; C-2 to H-1; C-3 to H-5, H-1, and OMe; C-4 to H-5; C-5 to H-1, H-7A/B; C6 to H1, H-7A/B; and C8 to H-7A/B confirmed

* To whom correspondence should be addressed. Tel: +44 1224 272895. Fax: +44 1224 272921. E-mail: m.jaspars@abdn.ac.uk.

10.1021/np020275n CCC: $22.00 © 2002 American Chemical Society and American Society of Pharmacognosy
Published on Web 10/22/2002

Table 1. ^{1}H and ^{13}C NMR Spectral Data in CD_3OD at 400/100 MHz for Compounds **2** and **5**

	compound **2**				compound **5**			
atom	^{13}C δ/ppm (mult)	^{1}H δ/ppm (H, mult, *J*/Hz)	COSY (^{1}H→^{1}H)	HMBC (^{13}C→^{1}H)	^{13}C δ/ppm (mult)	^{1}H δ/ppm (H, mult, *J*/Hz)	COSY (^{1}H→^{1}H)	HMBC (^{13}C→^{1}H)
1	75.2 (d)	4.04 (1H, s)		H5, H7A, H7B	134.4 (s)	7.40 (1H, d, 2.0)	H5, H7	H5, H7
2	113.8 (s)			H1	111.8 (s)			
3	148.9 (s)			H5, OMe	155.4 (s)			H1, H4, H5, OMe
4	122.5 (s)			H5	112.8 (d)	6.85 (1H, d, 8.0)	H5	
5	132.0 (d)	6.36 (1H, s)		H1, H7A, H7B	130.0 (d)	7.13 (1H, dd, 2.4, 8.4)	H1, H4,	H7
6	92.0 (s)			H1, H7A, H7B	131.4 (s)			H4, H7
7	39.0 (t)	A 3.73 (1H, d, 18.2)	H7B		28.3 (t)	3.76 (2H, s)	H1	
		B 3.01 (1H, d, 18.2)	H7A					
8	154.8 (s)			H7A, H7B	152.6 (s)			H7
9	161.2 (s)			H10	165.4 (s)			H7, H10
10	40.1 (t)	3.42 (2H, t, 7.6)	H11	H11	41.0 (t)	3.39 (2H, t, 7.2)	H11	
11	34.7 (t)	2.76 (2H, t, 7.6)	H10	H10, H13/H17	34.8 (t)	2.70 (2H, t, 6.9)	H10, H13/H17	
12	139.8 (s)			H10, H11	139.8 (s)			H10, H11
13	134.1 (d)	7.45 (1H, s)		H11	134.0 (d)	7.39 (1H, s)	H11	H11
14	118.4 (s)			H13	118.4 (s)			H13
15	151.9 (s)			H13/H17, H18	152.0 (s)			H13/H17, H18
16	118.4 (s)			H11, H17	118.4 (s)			
17	134.1 (d)	7.45 (1H, s)		H11	134.0 (d)	7.39 (1H, s)	H11	H11
18	71.4 (t)	4.08 (2H, t, 5.6)	H19	H19	70.9 (t)	4.03 (2H, t, 5.7)	H19	H19, H20
19	28.0 (t)	2.14 (2H, t, 6.0)	H18, H20	H18, H20	25.1 (t)	2.37 (2H, q, 6.4)	H18, H20	H18, H20
20	48.8 (t)	3.30 (3H, t, 6.6)	H19	NMe	69.0 (t)	3.61 (2H, 7.6, 8.4)	H19	H18, H19
OMe	60.1 (q)	3.69 (3H, s)			56.0 (q)	3.79 (3H, bs)		
NMe	32.0 (q)	2.69 (3H, s)						
$N^+O^-(Me)_2$					58.0 (q)	3.24 (6H, bs)		H20

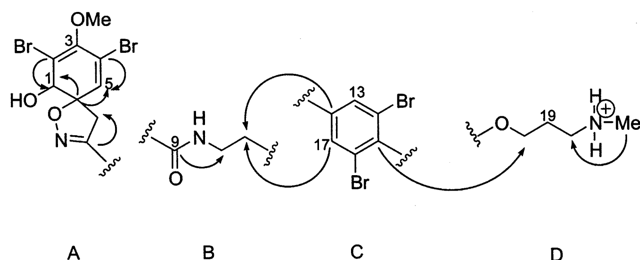


Figure 1. Substructures A–D of compound **2** and some key HMBC correlations.

the 1-hydroxy-2,4-dibromo-3-methoxy-8-carbonyl spirocyclohexadienyl isoxazole (partial structure A) system. The ^{1}H spectrum showed a signal of an isolated aryl proton (δ_H 7.45, 2H, bs), consistent with a symmetrically tetrasubstituted aromatic ring (substructure C). The connection between substructures A and C was obtained using HMBC correlations from the signals at δ_C 134.1 (C-13/C-17) and δ_C 139.8 (C-12) to a methylene group at δ_H 2.76 (2H, t, $J = 7.6$) that was assigned to H-11. The signal at δ_H 2.76 showed further coupling to a signal at δ_H 3.42 (2H, t, $J = 7.6$) assigned as H-10. The signal at δ_H 3.42 showed a long-range correlation to the amide carbon at δ_C 161.2 (C-9), suggesting that substructure B was an amide unit connected to two methylene groups. No HMBC correlation was detected between C9 and either of the two H-7 protons, but biogenetic arguments[4] and comparison to literature ^{13}C NMR data[3] suggested substructure B was linked to substructure A at C-8. Substructure D was established from ^{1}H–^{1}H COSY correlations between the methylene protons at δ_H 4.04 (2H, t, $J = 5.6$), 2.14 (2H, t, $J = 6.0$), and 3.30 (2H, t, $J = 6.6$). The methylene signal at δ_C 48.8 showed an HMBC correlation to an N-methyl group at δ_H 2.69 (3H, s), suggesting the presence of an $-O-CH_2-CH_2-CH_2-N^+H_2CH_3$ system (substructure D). The number of methyl groups attached to the ammonium ion was determined from the integration value in the ^{1}H spectrum. Connection between substructure C and substructure D was assigned by a long-range correlation between δ_C 151.9 (C-15) and the methylene group at δ_H 4.08 (H-18). The complete structure was confirmed from comparison of its ^{13}C NMR spectrum with those of other purealidines.[3] The *trans* geometry of the vicinal oxygen atoms at C-1 and C-6 was established by comparison of ^{13}C chemical shifts with other known compounds with the same spirocyclohexadienyl isoxazole bromo system.[21,38]

Interpretation the ^{13}C NMR spectrum of purpuramine J (**5**) indicated the presence of 23 carbons (Table 1), one O-methyl (δ_C 56.0), two ammonium-methyls (δ_C 58.0), six methylenes (δ_C 70.9, 69.0, 41.0, 34.8, 28.3, 25.1), five sp^2 methines (δ_C 134.4, 134.0, 134.0, 130.0, 112.8), and seven sp^2 quaternary carbons (δ_C 155.4, 152.0, 139.8, 131.4, 118.4, 118.4, 111.8) as well as an oxime (δ_C 152.6) and an amide carbonyl (δ_C 165.4). These accounted for all 23 carbons. The low-resolution mass spectrum displayed an isotopic cluster (663.97, 665.96, 667.98, 669.96) consistent with the presence of three bromine atoms. The high-resolution mass spectrum (HREIMS) gave an *m/z* of 663.9763 $[M + H]^+$ for a Δ 2.3 mmu calculated for $C_{23}H_{28}{}^{79}Br_3N_3O_5$. An unsaturation number of 10 was calculated, which in conjunction with six double bonds, one oxime, and one amide carbonyl group suggested the presence of two rings in the molecule.

Use of one- and two-dimensional NMR data (Table 1) enabled the construction of four substructures (Figure 2). The ^{1}H NMR spectra showed the presence of one spin system, consistent with a 1,2,4-trisubstituted benzene ring. These connections were supported by ^{1}H–^{1}H COSY correlations from H-1 to H-5, and H-4 to H-5 (substructure

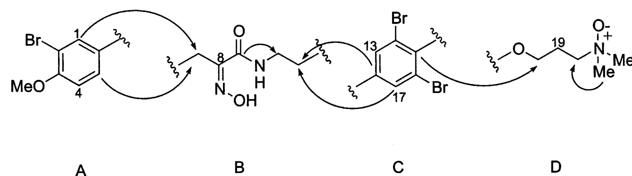


Figure 2. Substructures A–D of compound **5** and some key HMBC correlations.

Table 2. Cytotoxic Activity (ID_{50}, μg/mL) of Compounds **1**–**10**

no.	compound	A2780 (ovarian tumor)	K562 (leukaemia)
1	purealidin Q	2.54	1.49
2	purealidin S	7.44	6.02
3	aplysamine 2	2.83	1.37
4	purpureamine I	1.70	1.24
5	purpureamine J	6.77	5.97
6	aerophobin 2	>10.0	6.91
7	aerophobin 1	21.53	24.11
8	purealidin J	>10.0	>10.0
9	araplysillin 1	18.57	27.93
10	araplysillin 2	14.79	42.70

A). The presence of an amide and an oxime functionality was indicated by the IR absorbances at 1655 and 1620 cm^{-1} and ^{13}C resonances at δ_C 165.4 and 152.6 ppm, respectively. The ^1H NMR spectra of **5**, like **2**, showed a signal of an isolated aryl proton (δ_H 7.39, 2H, bs), consistent with a symmetrically tetrasubstituted aromatic ring (substructure C). The connection between substructures A and C was obtained by HMBC correlations from C-8 to H-7; C-9 to H-10; C-9 to H-7; C-12 to H-10; and C-12 to H-11. The partial structure from C-7 to C-11 was also ascertained from their ^{13}C chemical shifts, proton coupling constants, and $^1H-^1H$ COSY correlations between the methylene groups at C-10 and C-11. The partial structure from C-18 to C-20 was similar to compound **2** except at the terminal end, where there were two methyls attached to the terminal N-oxide group. The two N-methyls were determined from the integration value for the N-methyl signal at δ_H 3.24 (s) in the ^1H NMR spectrum of **5**, thus elucidating the presence of an $N^+(Me)_2$ group connected at C-20. The N-oxide ion is proposed, as both the proton and carbon resonances of the methylene group at C20 (δ_H 3.61 (2H, t), δ_C 69.0 (q)) showed strong deshielding indicative of the presence of a cationic nitrogen. Similar chemical shifts were observed with compounds containing the $-CH_2N^+O(CH_3)_2$ group in the literature[39,40] as well as compounds containing the $-CH_2N^+(CH_3)_3$ [41–43] or the $-CH_2N^+(CH_3)_2H$[34,44,45] moieties. The N-methyl groups and the methylene group at C-18 in the latter cases were found to resonate at a slightly higher field compared to compounds containing the N-oxide. The HREIMS data confirmed the presence of the oxide ion, as it was 16 amu exactly above the mass of aplysamine 2,[34] which can be envisaged as having lost a proton and subsequently gained an oxide ion to form compound **5**. The geometry of the C-8 oxime was elucidated as *E* from the chemical shift of C7.[11]

Compounds **2** and **5** showed moderate activity against ovarian tumor and leukemia cells, respectively (Table 2). Alkaloid N-oxides are considered rare from marine sources, although they are often isolated together with the corresponding free alkaloids from terrestrial sources.[39] This will be the fourth such report and the first bromotyrosine N-oxide containing compound. N-Oxides have been shown to be true natural products and not artifacts of separations by other workers.[39,40,46] Why the sponge synthesizes so many variations of the basic bromotyrosine skeleton type structure remains to be determined. Suggested roles for these bromotyrosine compounds are as feeding deterrents.[47]

Experimental Section

General Experimental Procedures. UV and IR were taken on a Perkin-Elmer Lambda 15 UV/vis spectrophotometer and Ati Mattson Genesis Series FTIR machine, respectively. ^1H, ^{13}C, and all NMR 2D experiments were recorded on a Varian Unity INOVA 400 MHz spectrometer, in CD_3OD solution. Low-resolution electrospray mass spectra were obtained on a Finnigan Masslab Navigator, and high-resolution mass data were obtained on a Finnigan MAT-95. HPLC separations were carried out using a Spectraseries P100 isocratic pump and monitored using a Hewlett-Packard HP 1050 Series variable-wavelength UV detector and a Waters reversed-phase (C_{18}, 10 × 250 mm) column.

Animal Material. The sample of *Druinella* sp. (order Verongida, family Druinellidae), collection number 9712SD060, was collected in December 1997 at a depth of about 5 m by snorkeling from Cakaulevu reef, in the district of Wainunu, in the island of Vanua Levu, Fiji Islands (17°2.609′; 178°54.694′ E). It was identified by John Hooper of the Queensland Museum, Australia. Voucher specimens are held at the South Pacific Herbarium, University of the South Pacific, Fiji, and at the Marine Natural Products Laboratory, University of Aberdeen, Scotland, U.K.

Extraction and Isolation. The freeze-dried sample (279.8 g dry weight) was extracted with MeOH (3×) and CH_2Cl_2 (3×), the solvent was removed under reduced pressure, and the extracts were combined. The crude oil was partitioned between water and CH_2Cl_2. The aqueous layer was then extracted with *s*-BuOH to give a brownish-red oil. The solvent was removed from the CH_2Cl_2 layer, and the resulting oil was partitioned between *n*-hexane and 10% aqueous MeOH. The MeOH layer was then phase adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2. The CH_2Cl_2 fraction was purified by Sephadex LH-20 chromatography (MeOH/CH_2Cl_2, 1:1) followed by reversed-phase flash chromatography using methanol as eluent. One pooled Sephadex fraction, S19–30, was found to contain interesting compounds on the basis of analysis of ^1H and ^{13}C NMR spectra. This fraction was purified by normal-phase silica HPLC using a mixture of dichloromethane, methanol, and ammonium hydroxide (80/20/1) as eluent to yield 9.8 mg of **1**, 6.7 mg of **2**, 31.3 mg of **3**, 5.7 mg of **4**, and 8.4 mg of **5**. The MeOH partition fraction was purified using the same solvent system to yield 7.7 mg of **6**, 6.8 mg of **7**, and 9.1 mg of **8**. The BuOH partition fraction was purified using the same solvent system, yielding 8.1 mg of **9**. In addition, a change of solvent to a mixture of acetonitrile and water (95/5) with a C_{18} HPLC column yielded 10.3 mg of compound **10**.

Purealidin S (2): colorless oil, 6.7 mg (0.0024% yield); UV (100% MeOH) λ_{max} 280 (log ϵ 3.28); IR ν_{max} (cm^{-1}) 1736, 1655, 1591, 1542, 1458, 1390, 1257, 1044, 737; NMR data (Table 1); LRESIMS *m/z* 726.85, 728.85, 730.85, 732.86, 734.85; [M + H]$^+$ HRESIMS *m/z* 728.8676 [M + H]$^+$ Δ 0.8 mmu calculated for $C_{22}H_{27}N_3O_5{}^{79}Br_3{}^{81}Br$.

Purpuramine J (5): colorless oil, 8.4 mg (0.0030% yield); UV (100% MeOH) λ_{max} 280 (ϵ 3.26); IR ν_{max} (cm^{-1}) 2937, 2852, 1743, 1656, 1533, 1493, 1452, 1253, 1047, 739; NMR data (Table 1); LRESIMS *m/z* 663.97, 665.96, 667.98, 669.96. [M + H]$^+$; HRESIMS *m/z* 663.9763 [M + H]$^+$ Δ 2.3 mmu calculated for $C_{23}H_{28}{}^{79}Br_3N_3O_5$.

Acknowledgment. We wish to thank Ratu I. Bulikiobo for permission to collect, U. Tabudravu, K. Ratuvuga, and I. Qativi for sponge collection, J. Hooper of Queensland Centre for Biodiversity for sponge identification, G. Duncan of the Rowett Institute for mass spectra, N. Smith of the Paterson Institute of Cancer Research, Manchester, for anticancer bioassays, the University of the South Pacific (USP) Marine Studies Programme and Department of Chemistry for use of facilities, and the USP, the Fiji Government, and ORS (U.K.) for funding.

References and Notes

(1) Capon, R. J.; Macleod, J. K. *Aust. J. Chem.* **1987**, *40*, 341−346.
(2) Minale, L.; Cimino, G.; DeStefano, S.; Sodano, G. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 1−72.
(3) Yagi, H.; Matsunage, S.; Fusetani, N. *Tetrahedron* **1993**, *49*, 3749−3754.
(4) Roll, M. D.; Chang, W. J. C.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van, D. G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 2916−2920.
(5) Ichiba, T.; Scheuer, P. J. *J. Org. Chem.* **1993**, *58*, 4149−4150.
(6) Copp, B. R.; Ireland, C. M. *J. Nat. Prod.* **1992**, *55*, 822−823.
(7) Kazlauskas, R.; Lidgrad, R. O.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Aust. J. Chem.* **1981**, *34*, 765−786.
(8) Pordesimo, O. E.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704−4709.
(9) Kazluskas, R. L. R, O.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1980**, *21*, 2277−2280.
(10) Carney, J. R.; Scheuer, P. J. *J. Nat. Prod.* **1993**, *56*, 153−157.
(11) Arabshahi, L.; Schmitz, F. J. *J. Org. Chem.* **1987**, *52*, 3584−3586.
(12) Jiminez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097−2102.
(13) Gunasekera, M.; Gunasekera, S. P. *J. Nat. Prod.* **1989**, *52*, 753−756.
(14) Cimino, G. R., S. de.; Stefano, S. de.; Self, R.; Sodano, G. *Tetrahedron Lett.* **1983**, *24*, 3029−3032.
(15) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Kobayashi, J. *Tetrahedron Lett.* **1992**, *33*, 2597−2598.
(16) Hirano, K.; Kubota, T.; Tsuda, M.; Watanabe, K.; Fromont, J.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 8107−8110.
(17) Gunasekera, S. P.; Cross, S. S. *J. Nat. Prod.* **1992**, *55*, 509−512.
(18) Kobayashi, J.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. *Tetrahedron* **1991**, *47*, 6617−6622.
(19) Andersen, R. J.; Faulkner, D. J. *Tetrahedron Lett.* **1973**, *14*, 1175−1178.
(20) Krejcarek, G. E.; White, R. H.; Hager, L. P.; McClure, W. O.; Johnson, R. D.; Rinehart, J. K. L. R.; Shaw, D. S.; Brusca, R. C. *Tetrahedron Lett.* **1975**, *8*, 507−510.
(21) Gao, H.; Kelly, M.; Hamann, M. T. *Tetrahedron* **1999**, *55*, 9717−9726.
(22) Longeon, A.; Guyot, M.; Vacelet, J. *Experientia* **1990**, *46*, 548−550.
(23) Nakamura, H.; Wu, H.; Kobayashi, J. *Tetrahedron Lett.* **1985**, *26*, 4517−4520.
(24) Wu, H.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Experientia* **1986**, *42*, 855−856.
(25) Okamoto, Y.; Ojika, M.; Kato, S.; Sakagami, Y. *Tetrahedron* **2000**, *56*, 5813−5818.
(26) Ross, S. A.; Weete, J. D.; Schinazi, R. F.; Wirtz, S. S.; Tharnish, P.; Scheuer, P. J.; Hamann, M. T. *J. Nat. Prod.* **2000**, *63*, 501−503.
(27) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1986**, *52*, 8181−8186.
(28) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Org. Chem.* **1996**, *61*, 2936−2937.
(29) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 1439−1440.
(30) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 8181−8186.
(31) Mierzwa, R. A., K.; Conover, M. A.; Tozzi, S.; Puar, M. S.; Patel, M.; Coval, S. J. *J. Nat. Prod.* **1994**, *57*, 175−177.
(32) Acosta, A. L.; Rodriguez, A. D. *J. Nat. Prod.* **1992**, *55*, 1007−1012.
(33) Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull.* **1995**, *43*, 403−407.
(34) Xynas, R.; Capon, R. J. *Aust. J. Chem.* **1989**, *42*, 1427−1433.
(35) Benharref, A.; Pais, M. *J. Nat. Prod.* **1996**, *59*, 177−180.
(36) Assman, M.; Wray, V.; Vansoest, R. W. M.; Proksch, P. *Z. Naturforsch. C Biosci.* **1998**, *53*, 398−401.
(37) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(38) Ciminiello, P.; Dell'Aversano, C.; Fattoruso, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **1999**, *62*, 590−593.
(39) Sato, A.; Fenical, W. *Tetrahedron Lett.* **1983**, *24*, 481−484.
(40) Gunawardana, G. P.; Kohmoto, S.; Burres, N. S. *Tetrahedron Lett.* **1989**, *30*, 4359−4362.
(41) Ciminiello, P.; Dell'aversano, C.; Fattorusso, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **2000**, *63*, 263−266.
(42) Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1999**, *62*, 1072−1073.
(43) Ciminiello, P.; Fatturosa, E.; Magno, S.; Pansini, M. *J. Nat. Prod.* **1994**, *57*, 1564−1569.
(44) Venkateswarlu, Y.; Venkatesham, U.; Rao, R. M. *J. Nat. Prod.* **1999**, *62*, 893−894.
(45) Venkateswarlu, Y.; Rao, M. N. A.; Venkatesham, U. *J. Nat. Prod.* **1998**, *61*, 1388−1389.
(46) Koren-Goldshlager, G.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *J. Org. Chem.* **1998**, *63*, 4601−4603.
(47) Koulman, A.; Proksch, P.; Ebel, R.; Beekman, A. C.; Uden, W. v.; Konings, A. W. T.; Pedersen, J. A.; Pras, N.; Woerdenbag, H. J. *J. Nat. Prod.* **1996**, *59*, 591−594.

NP020275N