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A NEW BASTADIN FROM THE SPONGE *PSAMMAPLYSILLA PURPUREA*

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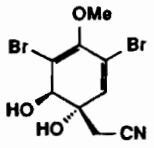
ABSTRACT.—A new bastadin **2** and the previously reported bastadins 5 [**3**], 7 [**4**], and 12 (formerly bastadin 9) [**5**] were isolated from the Verongid sponge *Psammaplysilla purpurea* collected in Pohnpei. Compound **2** is mildly cytotoxic against several cell lines and inhibits the enzymes topoisomerase II and dehydrofolate reductase.

Sponges of the order Verongida characteristically contain metabolites that are biogenetically derivable from halogenated tyrosine (1). These compounds range from aeroplysinin 1 [**1**] (2), which has recently been shown (J.R. Carney and K.L. Rinehart, manuscript in preparation) to be biosynthesized from tyrosine, monotyrosine, and dibromotyrosine (3), to the more elaborate macrocyclic bastadins, which are biogenetically derivable from four tyrosine units by oxidative phenolic coupling of two tyramine-tyrosine units (4–8). Bastadins have previously been reported only from *Ianthella basta*. We now report the isolation and structure of a new bastadin 14 [**2**], the major metabolite, and the previously reported bastadins 5 [**3**] (5), 7 [**4**] (5), and 12 (formerly 9) [**5**] (7), from the Verongid sponge *Psammaplysilla purpurea* (Carter) collected in Pohnpei, Federated States of Micronesia.

Previous studies of the bastadins were in press simultaneously (6–8), unfortunately leading to the assignment of the same number to different bastadins. We propose that the compounds be renumbered in the order in which they were received for publication. Thus, bastadins 8–11 of Pordesimo and Schmitz (6) retain their original numbering, bastadin 9 of Miao *et al.* (7) is renumbered as bastadin 12 [bastadin 8 from Miao *et al.* (7) coincidentally has the same structure as bastadin 8 of Pordesimo and Schmitz (6)], and bastadin 12 of Butler *et al.* (8) becomes bastadin 13.

The CH_2Cl_2-iPrOH (1:1) extract of the sponge showed moderate cytotoxicity against several cell lines. The extract was partitioned between hexanes-MeOH (2:1), and the MeOH layer was concentrated to a solid. Centrifugal countercurrent chromatography [EtOAc-heptane-MeOH-H_2O (7:4:4:3)] of the MeOH layer residue gave nearly pure **2** and **5**. Reversed-phase hplc of the fraction containing **2** removed trace amounts of **3** and **4** to yield pure **2**.

The molecular formula of **2** was $C_{34}H_{25}Br_5N_4O_8$ by hrfabms, indicating that **2** was isomeric with bastadin 4 [**6**], but its ^{1}H- and ^{13}C-nmr spectra were significantly different from those reported for **6** (5,6). ^{1}H-^{1}H nmr decoupling experiments of the downfield region indicated the presence of one symmetric and two unsymmetric tetrasubstituted aromatic rings, and one trisubstituted aromatic ring. The ^{1}H-nmr spectrum (DMSO-d_6) of **2** showed exchangeable proton signals at δ 12.10 (2H, bs), 11.85 (1H, s), 10.31 (1H, d, J = 10.2 Hz), 9.87 (1H, bs), and 7.80 (1H, t, J = 5.7 Hz), and the ^{13}C-nmr spectrum displayed all 34 carbon signals (Table 1). HMQC and HMBC nmr experiments established C-H and C-C connectivities. A doublet at δ 10.31 showed H-C correlations to alkene (δ 110.0, C-6) and amide carbonyl (161.4, C-3) carbon signals and was coupled to a one-proton doublet of doublets at δ 7.45. The doublet of doublets, which collapsed to a single doublet (J = 14.3 Hz) when the



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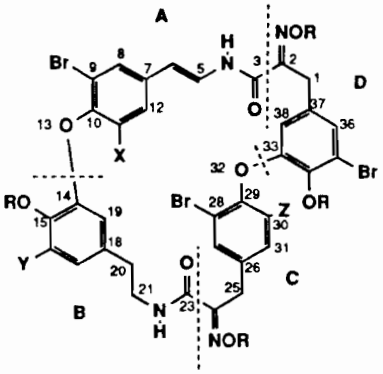
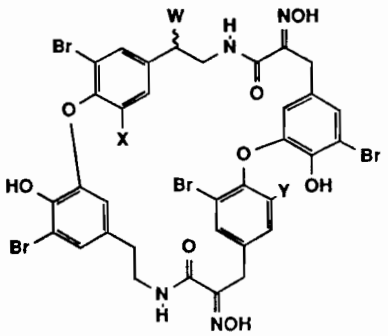
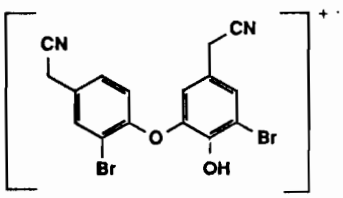


2 X=Br, Y=Br, Z=H, R=H
4 X=H, Y=Br, Z=H, R=H
6 X=H, Y=Br, Z=Br, R=H
7 X=H, Y=H, Z=Br, R=H
9 X=Br, Y=Br, Z=H, R=Me



3 W=H, X=H, Y=Br
5 W=OH, X=Br, Y=H



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spectrum was determined in MeOH-d_4, was in turn coupled to a one-proton doublet at δ 6.41. These data indicated an eneamide system similar to that in bastadins 4 [**6**], 7 [**4**], and 11 [**7**], but the proton at δ 6.41 showed a correlation to a two-carbon signal at δ 129.5 (C-8 and -12), in addition to correlations to the alkene and aromatic carbons at δ 126.0 (C-5) and 137.6 (C-7). H-C correlations of a two-proton singlet at δ 7.77 to carbon signals at δ 110.0 (C-6), 118.1 (2C's, C-9 and -11), 129.5 (C-8 and -12), and 145.6 (C-10) firmly established the structure of the A portion.

H-C correlations were observed between a broad two-proton singlet at δ 3.70, typical for benzylic protons adjacent to an oxime of the bastadins (4–8), to the α-oximino amide carbons (C-2 and -3) of the upper portion of the molecule. The benzylic protons also showed correlations to aromatic carbon signals at δ 128.3 (C-37) and 117.3 (C-38). A doublet at δ 7.20, meta-coupled to a broad singlet at δ 6.40, showed an H-C correlation to C-38, as well as to a carbon signal at δ 143.8 (C-34). The proton at δ 6.40 also showed a correlation to C-34 and to C-33 (δ 144.8), thus establishing the 1,3,4,5 substitution pattern of the D subunit and hence the structure of the upper portion of the molecule.

TABLE 1. Nmr Data for **2** (DMSO-d_6).

Position	^{13}C	^{1}H (J, Hz)	HMBC
1	27.5	3.70, 2H, s	
2	151.5		
3	161.4		4, 5
4		10.31, 1H, d (10.2)	
5	126.0	7.45, 1H, dd (14.3, 10.2)	4, 6
6	110.0	6.41, 1H, d (14.3)	4, 8, 12
7	137.6		5, 6, 8, 12
8	129.5	7.77, 1H, s	6
9	118.1		8, 12
10	145.6		8, 12
11	118.1		8, 12
12	129.5	7.77, 1H, s	6
14	145.0		
15	141.6		17, 19
16	110.1		17
17	126.4	7.00, 1H, d (1.5)	20
18	130.8		20, 21
19	111.7	6.18, 1H, d (1.5)	17
20	32.8	2.67, 2H, t (6.5)	
21	38.3	3.19, 2H, q (6.3)	20, 22
22		7.80, 1H, t (5.7)	
23	162.9		25
24	150.9		25
25	28.4	3.59, 2H, s	
26	134.4		25, 30
27	133.7	7.46, 1H, bs	31
28	112.8		27
29	150.9		27, 30, 31
30	119.1	6.64, 1H, d (8.3)	
31	130.3	7.14, 1H, dd (8.3, 1.9)	27
33	144.8		
34	143.8		36, 38
35	110.9		
36	126.0	7.20, 1H, d (1.9)	
37	128.3		1
38	117.3	6.40, 1H, bs	1, 36

A broad two-proton singlet at δ 3.59 arising from the benzylic protons adjacent to the other oxime showed H-C correlations to the α-oximino amide carbon signals of the lower portion of the molecule at δ 150.9 (C-24) and 162.9 (C-23), as well as to carbon signals at 130.3 (C-31) and 134.4 (C-26). A one-proton doublet at 6.64 (J = 8.3 Hz), ortho-coupled to a proton at δ 7.14, which was in turn meta-coupled to a proton at δ 7.46 (J = 1.9 Hz), also showed a correlation to C-26. Further H-C correlations confirmed the structure of the C portion.

An exchangeable triplet at δ 7.80 showed correlations to the amide carbonyl (δ 162.9, C-23) and to a carbon signal at δ 38.3 (C-21). A two-proton triplet at δ 2.67 also showed a correlation to this carbon and to the aromatic carbon signals of subunit B at 130.8 (C-18) and 126.4 (C-17). A broad two-proton quartet at δ 3.19, which collapsed to a triplet in MeOH-d_4, showed correlations to C-23 and a three-bond correlation to C-18, thus connecting the B and C subunits. The remaining aromatic protons were meta-coupled (1.5 Hz), and both showed 3-bond correlations to

C-15, but only the proton attached to C-19 showed a correlation to C-14, thus securing the carbon assignments and substitution pattern of the aromatic ring of the B subunit.

The carbon chemical shifts of C-1 (δ 27.5) and C-25 (δ 28.4) implied that both oximes had *E* geometry (9).

With the connectivities of the A to D and B to C subunits established, ms was used to secure the connectivity of C to D. An eims of **2** gave an ion fragment cluster at *m/z* 420/422/424 (1:2:1); hreims of the ion at *m/z* 422 indicated a formula of $C_{16}H_{10}{}^{79}Br^{81}BrN_2O_2$, attributable to the radical cation **8** (4–8).

Although it was reasonable that the ether linkages between the A and B and between the C and D rings were from C-10 to C-14 and C-29 to C-33, the recent report of bastadin 13 (formerly 12) (8), with rings A and B linked from C-9 to C-14, plus discrepancies between our carbon chemical shift assignments and those reported for the same subunits by Pordesimo and Schmitz (6) led us to investigate further the ether linkages of **2**. Compound **2** was converted to its tetramethyl ether **9** by treatment with MeI and K_2CO_3 in DMF (4–7), and HMQC and HMBC experiments established the C-H and C-C connectivities of **9**. The two *O*-methyl groups at δ 3.83 and 4.03 showed only the expected three bond H-C correlations to C-34 (δ 147.5) and C-15 (δ 144.3). Both protons attached to the aromatic ring of subunit B showed strong three-bond H-C correlations to C-15, but only the proton attached to C-19 showed a strong correlation to C-14 (δ 150.4). Similarly, both aromatic protons in D showed H-C correlations to C-34, and only the proton attached to C-38 showed a correlation to C-33 (δ 148.9). These data confirmed the total structure of **2**

The possibility that **2** is an artifact derived from dehydration of **5** during isolation can be excluded since we employed mild conditions which are unlikely to cause dehydration and since the alkene signals of **2** at δ 6.41 and 7.45 were clearly detectable in the ^{1}H-nmr spectrum of the crude extract. In fact, compound **5** was quite stable; stirring the compound for 2 weeks in MeOH with TFA provided no **2**, as evidenced by ^{1}H nmr.

Compound **2** was mildly cytotoxic against several cell lines. IC_{50} values of 2 μg/ml were determined in assays against A-549 lung carcinoma, HT-29 colon adenocarcinoma human tumor, and the P-388 murine lymphocytic leukemia cell lines, and 2.5 μg/ml against the nontumor CV-1 monkey kidney cell line. The compound also inhibited the enzymes topoisomerase-II and dehydrofolate reductase, with IC_{50} values of 2.0 and 2.5 μg/ml, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer Model 1420 spectrophotometer and uv spectra on a Hewlett-Packard Model 8452A diode array detector. Nmr spectra were measured on a General Electric GN500 at 500 MHz (^{1}H) and 125 MHz (^{13}C). Mass spectra were provided by the University of Illinois mass spectrometry facility. A P.C. Inc., multilayer coil separator-extractor was used for centrifugal countercurrent chromatography at a flow rate of 3 ml/min at 800 rpm with a no. 10 column (380 ml volume). YMC 5 μ ODS and Rainin Si gel 10 × 250 mm columns were used for the hplc separations.

TAXONOMY.—The sponge was collected on June 21, 1990 from a cave at a depth of 3–5 m in Pingelap Atoll, Pohnpei, Federated States of Micronesia. The sponge formed a thin encrustation with a conulose surface. The color in life was green and turned deep purple after exposure to air. The sample corresponds most closely to *P. purpurea* (Order Verongida, Family Aplysinellidae) as described by Bergquist (10) and Kelly-Borges and Bergquist (11). A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (catalog number 003:00596).

ISOLATION.—The freeze-dried sponge (7.3 g) was extracted overnight with 200 ml of CH_2Cl_2-iPrOH (1:1), and the residue (0.585 g) was partitioned between 200 ml hexanes-MeOH (2:1); the MeOH layer yielded 0.457 g of a red solid. The MeOH layer residue was subjected to centrifugal countercurrent chromatography using EtOAc-heptane-MeOH-H_2O (7:4:4.3), with the

upper phase as the mobile phase, yielding 96 mg of nearly pure **2** and 33 mg of **5**. Crude **2** was separated from trace amounts of **3** and **4** by reversed-phase hplc [ODS, MeCN-H_2O (56:44), 3 ml/min].

Bastadin 14 [**2**].—Hrfabms *m/z* $[M + H]^+$ 1018.7613 (calcd for $C_{34}H_{26}{}^{79}Br_2{}^{81}Br_3N_4O_8$, 1018.7601); hreims *m/z* 421.9090 (calcd for $C_{16}H_{10}{}^{79}Br^{81}BrN_2O_2$, 421.9089); eims (rel. int.) 424 (2.4), 422 (4.5), 420 (2.5), 316 (3.4), 314 (2.6), 303 (2.7), 301 (3.1); ir ν max (Nujol) 3500–3300, 1655, 1580, 1530, 1500, 1455, 1425, 1280, 1245, 1230, 1180 cm^{-1}; uv λ max (MeOH) nm (log ϵ) 284 (4.3), 292 (4.3), 314 (4.4); ^1H and ^{13}C nmr see Table 1.

Bastadin 14 tetramethyl ether [**9**].—Compound **9** was prepared as described (7) from 12 mg of **2**. Si gel hplc [hexanes-EtOAc (1:1), 3 ml/min] of the reaction mixture gave 6 mg of **12**: fabms *m/z* $[M + H]^+$ centered at 1074.9; ^{13}C nmr ($CDCl_3$) δ 162.3 (C-23), 159.6 (C-3), 152.1 (C-29), 150.9 (C-2), 150.4 (C-24), 150.4 (C-14), 148.9 (C-33), 147.5 (C-34), 146.4 (C-10), 144.3 (C-15), 136.9 (C-7), 135.5 (C-18), 134.1 (C-27), 133.0 (C-37), 132.5 (C-26), 129.9 (C-31), 129.9 (C-36), 129.8 (C-8 and C-12), 126.7 (C-17), 124.8 (C-5), 121.3 (C-38), 118.5 (C-9 and C-11), 118.1 (C-16), 117.9 (C-35), 117.1 (C-30), 112.5 (C-19), 112.3 (C-28), 110.0 (C-6), 63.7 (MeON), 63.1 (15-OMe), 61.1 (34-OMe), 61.0 (MeON), 39.4 (C-21), 34.5 (C-20), 29.2 (C-25), 28.3 (C-1), ^1H nmr ($CDCl_3$) δ 8.44 (1H, d, J = 8.4 Hz, NH-4), 7.57 (2H, s, H-8, -12), 7.47 (1H, d, J = 2.2 Hz, H-27), 7.46 (1H, dd, J = 14.6, 8.4 Hz, H-5), 7.32 (1H, d, J = 2.2 Hz, H-36), 7.12 (1H, dd, J = 8.4, 2.2 Hz, H-31), 7.03 (1H, d, J = 2.0, H-17), 6.86 (1H, d, J = 2.0 Hz, H-38), 6.71 (1H, t, J = 6.5 Hz, NH-22), 6.47 (1H, d, J = 8.4 Hz, H-30), 6.15 (1H, d, J = 2.0, H-19), 6.11 (1H, d, J = 14.6, H-6), 4.10 (3H, s, NOMe), 4.05 (3H, s, NOMe), 4.03 (3H, s, 15-OMe), 3.85 (2H, bs, H-1), 3.83 (3H, s, 34-OMe), 3.75 (2H, bs, H-25), 3.36 (2H, bq, J = 6.5 Hz, H-21), 2.72 (2H, t, J = 6.5 Hz, H-20).

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