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ISOLATION OF A NOVEL BASTADIN FROM THE TEMPERATE MARINE SPONGE *IANTHELLA* SP.

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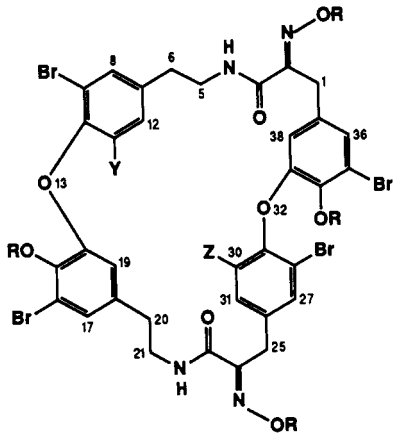
ABSTRACT.—A novel secondary metabolite, bastadin 15 [**1**], has been isolated from a temperate *Ianthella* sp. The sponge also yielded four bastadins previously isolated from the tropical species *Ianthella basta*.

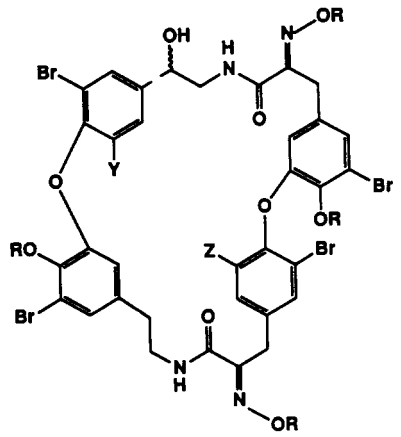
The bastadins, macrocyclic derivatives of bromotyrosine, were first isolated from the tropical verongid sponge *Ianthella basta* by Kazlauskas and coworkers (1,2), and later by Pordesimo and Schmitz (3). In the light of recent reports (4–6) of novel bastadins from this species, we present results from a temperate *Ianthella* sp. collected at Pig Island, New South Wales. We here report isolation of a novel member of the group, bastadin 15 [**1**], and full characterization of the derivative tetramethylbastadin 15 [**2**]. Also present in this sponge are the known compounds bastadin 8 [**3**] and bastadin 12 [**4**], here fully characterized for the first time (via the derivatives tetramethylbastadin 8 [**5**] and tetramethylbastadin 12 [**6**]), and bastadins 4 [**7**] and 6 [**8**]. [See Carney *et al.* (6) for a discussion on the numbering of bastadin metabolites.]

Samples of *Ianthella* sp. (order Verongidae) were collected by hand using scuba at a site near Pig Island, New South Wales, Australia. The MeOH-soluble fraction from an EtOH extract of the sponge was subjected to flash chromatography on silica to yield a crude bastadin fraction which was separated using reversed-phase hplc to yield, in addition to four known bastadins, the novel compound bastadin 15 [**1**].

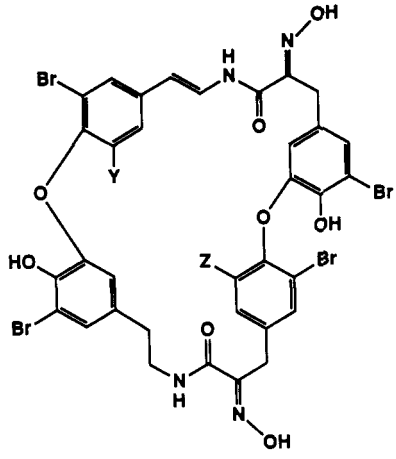
Bastadin 15 [**1**] (33 mg) was isolated as a white amorphous solid with very weak optical activity. Inspection of the



1 Y=Br, Z=R=H
2 Y=Br, Z=H, R=Me
8 Y=Z=Br, R=H
9 Y=R=H, Z=Br



3 Y=R=H, Z=Br
4 Y=Br, Z=R=H
5 Y=H, Z=Br, R=Me
6 Y=Br, Z=H, R=Me



7 Y=H, Z=Br
10 Y=Br, Z=H

^{1}H-nmr spectrum (Table 1) showed the compound to be a bastadin. Hrfabms gave a molecular ion cluster at *m/z* 1014–1024, corresponding to $C_{34}H_{27}N_4O_8Br_5$. 1D proton decoupling experiments and COSY-90 showed two aromatic rings with asymmetric 1,2,3,5-tetrasubstitution, one 1,2,3,5 symmetrically substituted ring, and one 1,2,4 trisubstituted ring. Difference nOe and NOESY experiments gave the connectivities of the aromatic rings with the aliphatic sidechains and hence the tentative structure **1**, by analogy to the known secondary metabolite bastadin 5 [**9**]. It then remained to determine the amide and ether linkages between the tyrosine-derived units.

TABLE 1. ^{1}H-nmr Assignments of Bastadin 15 [**1**].[a]

Proton	δ ^{1}H
H-1	3.71 (s)
H-4	6.76 (t, J = 6.2 Hz)
H-5	3.51 (m)
H-6	2.82 (m)
H-8	7.38 (s)
H-12	7.38 (s)
H-17	7.04 (d, J = 1.9 Hz)
H-19	6.14 (d, J = 1.9 Hz)
H-20	2.67 (m)
H-21	3.34 (m)
H-22	7.16 (t, J = 6.4 Hz)
H-25	3.88 (s)
H-27	7.49 (d, J = 2.0 Hz)
H-30	6.87 (d, J = 8.3 Hz)
H-31	7.14 (dd, J = 2.0, 8.3 Hz)
H-36	7.22 (d, J = 2.0 Hz)
H-38	6.52 (d, J = 2.0 Hz)
Oxime	11.48 (bs)
Phenol	10.17 (bs)

[a]Solution in $CDCl_3$ with one drop DMSO-d_6. Referenced to internal TMS; 400 MHz. Assignments based on ^{1}H-^{1}H decoupling and difference nOe experiments.

To overcome the limited solubility of the pure compounds and facilitate complete characterization, samples of the purified bastadins were treated with MeI in Me_2CO to yield permethylated derivatives. Tetramethylbastadin 15 [**2**] (45 mg) was an amorphous white solid. Hrfabms gave a molecular ion cluster at *m/z* 1070–1080 corresponding to $C_{38}H_{35}N_4O_8Br_5$. Initial assignment of the ^{1}H-nmr spectrum of **2** was carried out by COSY-90 and NOESY experiments. HMQC and HMBC ^{1}H-^{13}C correlation studies allowed the assignment of the ^{13}C spectrum (Table 2). In particular, correlations from the amide carbonyl C-3 to H-4 and methylene protons H-1 and H-5, and similar correlations from C-23 to H-22, H-21, and H-25 gave the ordering of partial structures in the macrocyclic ring system. The two *O*-methyl signals gave three-bond correlations to C-15 and C-34; H-17 and H-19 both showed correlations to C-15, but only H-19 showed correlations to C-14. Similar arguments confirmed the nature of the ether linkages in ring D. These assignments of tetramethylbastadin 15 [**2**] are similar to the spectral assignments given by Pordesimo and Schmitz (3) for the isomeric bastadin 5 [**9**] and by Carney *et al.* (6) for bastadin 14 [**10**]. We thus assigned **1** as based on the 13,32-dioxa-4,22-diazabastarane macrocycle known to be contained in bastadins 4 [**7**], 6 [**8**], and 8 [**3**] which are also present in this sponge, rather than on the 3,32-dioxa-4,22-diazaisobastarane suggested by Butler *et al.* (4) for bastadin 13.

Bastadins 8 [**3**] and 12 [**4**] were iden-

TABLE 2. ^{13}C- and ^{1}H-nmr Assignments of Tetramethylbastadin 15 [2].

Position	δ ^{13}C[a]	δ ^{1}H[b]
1	28.6	3.78 (s, 2H)
2	151.2[c]	—
3	162.0	—
4	—	6.80 (t, J = 5.9 Hz)
5	40.2	3.55 (m, 2H)
6	34.7	2.78 (m, 2H)
7	139.4	—
8	133.6	7.42 (s)
9	118.2	—
10	146.7	—
11	118.2	—
12	133.6	7.42 (s)
14	150.2	—
15	144.6	—
16	118.4	—
17	126.4	7.05 (d, J = 2.1 Hz)
18	135.3	—
19	113.0	6.15 (d, J = 2.1 Hz)
20	34.9	2.65 (m, 2H)
21	39.9	3.38 (m, 2H)
22	—	6.76 (t, J = 5.8 Hz)
23	162.1	—
24	151.1[c]	—
25	28.9	3.81 (s, 2H)
26	133.2[d]	—
27	134.2	7.50 (d, J = 2.1 Hz)
28	113.8	—
29	151.7	—
30	119.1	6.66 (d, J = 8.3 Hz)
31	129.6	7.12 (dd, J = 2.1, 8.3 Hz)
33	149.8	—
34	146.6	—
35	117.8	—
36	129.0	7.27 (d, J = 2.1 Hz)
37	133.2[d]	—
38	119.0	6.64 (d, J = 2.1 Hz)
OMe	61.0	4.03 (s)
	63.1	4.01 (s)
	61.1	3.89 (s)
	62.9	3.73 (s)

[a]Solution in $CDCl_3$ referenced to $CHCl_3$ at 77.0 ppm; 100 MHz. Assignments based on HMQC and HMBC ^{1}H-^{13}C correlation experiments at 500 MHz.

[b]Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 7.25; 400 MHz. Assignments based on ^{1}H-^{1}H decoupling and difference nOe experiments.

[c]Assignments interchangeable.

[d]2C coincidental overlap.

tified indirectly using ^{1}H-nmr spectra of the tetramethylated derivatives **5** and **6** (5). A direct identification of the unmodified natural products was difficult due to poor reproducibility of spectra in the mixed solvents reported in the literature ($CDCl_3$/DMSO-d_6) (5). Full characterization of these compounds was also carried out using the methylated derivatives. The ^{1}H spectral assignments obtained by us from COSY-90 and NOESY experiments are in agreement with those reported by Miao *et al.* (5). ^{13}C spectrum assignment was carried out using HMQC and HMBC ^{1}H-^{13}C correlations as for tetramethylbastadin 15.

Bastadin 4 [7] was identified by comparison of ^{1}H- and ^{13}C-nmr spectra to literature (2,3). Bastadin 6 [8] was identified by comparison of the ^{1}H-nmr spectrum to literature data (2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—These have been reported previously (7).

MATERIALS.—Si gel 60 was from Merck (Darmstadt). DMSO-d_6, $CDCl_3$, and MeOH-d_6 were from Sigma (St Louis). DMSO-d_6 was allowed to stand over vacuum-dried 3 Å molecular sieves for at least 24 h before use. Nmr spectra were recorded using a Jeol GX-400, Bruker AC-200F or Bruker AMX-500 spectrometer. Solvents were distilled from laboratory or analytical grade reagents before use. Hrfabms data were acquired on a VG 70-VSE spectrometer by voltage scanning at m/Δm 7000 (10% valley).

SPONGE.—Sponge was collected by hand using scuba at −15 to −22 m at Pig Island, New South Wales, Australia and was stored frozen at −20° before extraction. *Ianthella* sp. is typically fan-shaped and of a yellow color when alive, turning purple-black following collection. It has a highly developed fibrous skeleton characterized by secondary extension of the skeleton at right angles to the primary fan. The taxonomy of this genus is currently under revision (P.R. Bergquist, personal communication). A voucher specimen (#G301211) of the sponge has been lodged at the Queensland Museum, Grey St., South Brisbane.

ISOLATION.—Thawed sponge (450 g wet wt) was extracted with 90% aqueous EtOH (3 × 3 liters) at −20°. The combined extracts were taken to dryness, and the residue (16.4 g) was triturated sequentially with CH_2Cl_2 (1 liter) and MeOH (1 liter). The MeOH extract (14.0 g) was subjected to normal-phase flash chromatography. Crude sponge extract (1.0 g) was dissolved in MeOH

and adsorbed onto flash Si gel 60 (1.0 g) before loading onto a silica column pre-equilibrated with CH_2Cl_2. Single-step elution with CH_2Cl_2-MeOH (15:1) gave a crude bastadin fraction (113 mg) which was substantially free of pigments. Bastadin fraction (230 mg) was purified by reversed-phase hplc [MeOH-H_2O (66:34)] to give bastadin 15 [**1**] (33 mg), bastadin 4 [**7**] (27 mg), bastadin 6 [**8**] (73 mg), and a mixture of bastadins 8 [**3**] and 12 [**4**] (40 mg).

METHYLATION.—In a typical procedure, a mixture (40 mg) of **3** and **4** was dissolved in Me_2CO (30 ml) and treated with excess MeI in the presence of K_2CO_3 (1.2 g) for 24 h at room temperature. The reaction mixture was dried in vacuo, resuspended in H_2O, and partitioned against $CHCl_3$. Purification of the $CHCl_3$-soluble portion by normal phase hplc [EtOAc-hexane (26:74)] yielded **5** (15 mg) and **6** (9 mg). Similarly, bastadin 15 [**1**] was treated with MeI to yield **2**. Final purification of methylated bastadins was carried out by reversed-phase hplc [MeOH-H_2O (90:10)].

Bastadin 4 [**7**] *(27 mg) and bastadin 6* [**8**] *(73 mg).*—White amorphous solids: ^{1}H- and ^{13}C-nmr data for **7** and ^{1}H-nmr data for **8** were in agreement with literature (2,3).

Bastadin 15 [**1**] *(33 mg).*—White amorphous solid: [α]D negative, but too small to be measured accurately; ^{1}H nmr see Table 1; hrfabms *m/z* $[M+H]^+$ 1016.7785 (calcd 1016.7803 for $C_{34}H_{28}N_4O_8{}^{79}Br_4{}^{81}Br$).

Tetramethylbastadin 8 [**5**] *(15 mg).*—White amorphous solid: [α]D +6.8 ($c=0.647$, CH_2Cl_2); ^{1}H and ^{13}C nmr in agreement with literature (5); ^{13}C nmr see Table 3; hrfabms *m/z* $[M+H]^+$ 1086.8354 (calcd 1086.8399 for $C_{38}H_{36}N_4O_9{}^{79}Br_5$).

Tetramethylbastadin 12 [**6**] *(9 mg).*—White amorphous solid: [α]D −4.4 ($c=0.826$, CH_2Cl_2); ^{1}H- and ^{13}C-nmr spectra in agreement with literature (5); ^{13}C nmr see Table 3; hrfabms *m/z* $[M+H]^+$ 1086.8423 (calcd 1086.8399 for $C_{38}H_{36}N_4O_9{}^{79}Br_5$).

Tetramethylbastadin 15 [**2**] *(45 mg).*—White amorphous solid: [α]D −0.8 ($c=3.613$, CH_2Cl_2); uv ($CHCl_3$) λ max 242 (log ε 4.3); ^{1}H and ^{13}C nmr see Table 2; hrfabms *m/z* $[M+H]^+$ 1070.8482 (calcd 1070.8450 for $C_{38}H_{36}N_4O_8{}^{79}Br_5$).

TABLE 3. ^{13}C-nmr Assignments of Tetramethylbastadin 8 [**5**] and Tetramethylbastadin 12 [**6**].[a]

Carbon	5	6
C-1	28.6	28.6
C-2	150.3[b]	151.1[d]
C-3	163.3	163.6
C-5	47.1	47.3
C-6	72.4	72.2
C-7	139.2	142.1
C-8	131.2	130.6
C-9	114.6	117.8
C-10	152.4	147.5
C-11	120.1	117.8
C-12	126.6	130.6
C-14	150.2[b]	150.2
C-15	146.5	144.5
C-16	118.1	118.6
C-17	127.9	126.6
C-18	135.6	135.3
C-19	117.8[c]	112.7
C-20	35.0	34.8
C-21	39.7	39.6
C-23	162.2	162.3
C-24	150.9	150.8[d]
C-25	29.1	29.0
C-26	136.4	133.1[e]
C-27	133.7	134.2
C-28	118.0	113.4
C-29	146.8	151.8
C-30	118.0	118.3[g]
C-31	133.7	129.7
C-33	149.9	149.6
C-34	144.5	146.8
C-35	117.9[c]	118.3[g]
C-36	127.5	129.2
C-37	132.7	133.1[e]
C-38	114.0	119.5
OMe	63.3	63.1[f]
	62.8	61.1
	61.2	61.0
	60.9	—

[a]Solution in $CDCl_3$ referenced to $CHCl_3$ at 77.0 ppm; 100 MHz. Assignments based on HMQC and HMBC ^{1}H-^{13}C correlation experiments at 500 MHz.

[b–d]Assignments interchangeable.

[e–g]2C coincidental overlap.

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LITERATURE CITED

1. R. Kazlauskas, R.O. Lidgard, P.T. Murphy, and R.J. Wells, *Tetrahedron Lett.*, **21**, 2277 (1980).
2. R. Kazlauskas, R.O. Lidgard, P.T. Mur-

phy, R.J. Wells, and J.F. Blount, *Aust. J. Chem.*, **34**, 765 (1981).
3. E.O. Pordesimo and F.J. Schmitz, *J. Org. Chem.*, **55**, 4704 (1990).
4. M.S. Butler, T.K. Lim, R.J. Capon, and L.S. Hammond, *Aust. J. Chem.*, **44**, 287 (1991).
5. S. Miao, R.J. Andersen, and T.M. Allen, *J. Nat. Prod.*, **53**, 1441 (1990).
6. J.R. Carney, P.J. Scheuer, and M. Kelly-Borges, *J. Nat. Prod.*, **56**, 153 (1993).
7. M.J. Garson, D.C. Manker, K.E. Maxwell, B.W. Skelton, and A.H. White, *Aust. J. Chem.*, **42**, 611 (1989).

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