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## ISOLATION AND STRUCTURE ELUCIDATION OF 34-SULFATOBASTADIN 13, AN INHIBITOR OF THE ENDOTHELIN A RECEPTOR, FROM A MARINE SPONGE OF THE GENUS *IANTHELLA*

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**ABSTRACT.**—34-Sulfatobastadin 13 [1] was isolated from the sponge *Iantbella* sp. Its structure was elucidated by nmr techniques and chemical transformation to bastadin 13 [2]. Compound 1 weakly inhibited binding to the endothelin A receptor (ET<sub>A</sub>), while compound 2 inhibited growth of *Bacillus subtilis*.

Sponges of the genus *Iantbella* have been reported to contain bromotyrosine derivatives (1–5). These compounds possess variable numbers of tyrosine units as well as different substitution patterns on the tyrosine units which give rise to different chain lengths and ring sizes. Most of these metabolites have been reported to be potent antimicrobial agents (4). The compounds bastadin 1 through bastadin 7 were first reported by Kazlauskas *et al.* (4). Their structures were confirmed by chemical derivatization and X-ray crystallography. The bastadins 1, 2, and 3 and bastadin 6 trimethyl ether have been synthesized by Nishiyama and co-workers (6,7). In a recent report Pordesimo and Schmitz (8) added bastadin 8 through bastadin 11 to the series. Their structures were derived by extensive nmr studies and comparison with the published data. The two most recent reports on the bastadins indicate the presence of bastadin 13 (previously called bastadin 12) (9), which possesses a different ring pattern with an alternative oxidative cyclization of the general bastadin leading to a novel series, and bastadin 14 (10). [Bastadin 9 of Miao *et al.* (11) and bastadin 12 of Butler *et al.* (9) were renamed bastadin 12 and bastadin 13, respectively, by Carney *et al.* (10).] During our bioassay-guided investigation of an *Iantbella*

sp. collected from the Great Barrier Reef in Australia, we isolated 34-sulfatobastadin 13 as the first sulfated compound of the bastadin series. During the preparation of this manuscript we found that J.A. Chan *et al.* (SmithKline Beecham Pharmaceuticals) have also isolated the same compound from *Iantbella flabelliformis* (Pallas) as an ATP Citrate Lyase inhibitor (ASP meeting abstract #P-29, Colonial Williamsburg, Virginia, July 26–31, 1992).

The sponge *Iantbella* sp. was collected at a depth of 9 m using scuba in the far northern region of the Great Barrier Reef in Australia, and stored frozen at –20° until the isolation started. The wet sponge was extracted with EtOH, blended, and filtered. The EtOH extract was concentrated to dryness and partitioned between *n*-BuOH and H<sub>2</sub>O. Chromatography of the *n*-BuOH partition on Sephadex LH-20 followed by Si gel cc yielded compound 1. Negative ion high resolution fabms of 1 gave the molecular formula of C<sub>34</sub>H<sub>27</sub><sup>79</sup>Br<sub>2</sub><sup>81</sup>Br<sub>2</sub>N<sub>4</sub>O<sub>11</sub>S [M–Na]<sup>–</sup>, indicating the presence of sulfur in the molecule. The <sup>1</sup>H- and <sup>13</sup>C-nmr studies on the molecule along with COSY, DEPT, HMQC, HMBC (see Figure 1 for connectivities) and comparison of the literature data (8) led us to assign the partial structures A–D. The partial

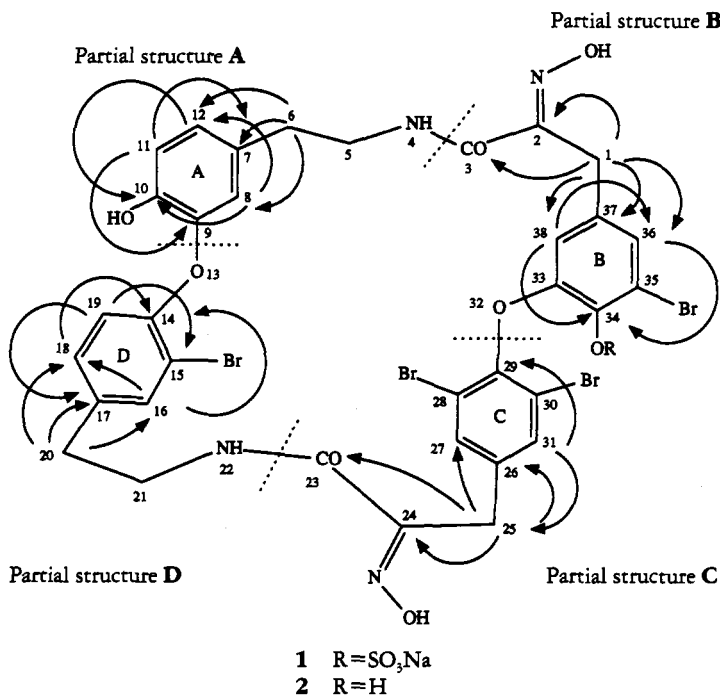


FIGURE 1. Long-range  $^1\text{H}/^{13}\text{C}$  correlations shown by the HMBC experiment on 34-sulfatobastadin 13 [1].

structures **A/B** and **C/D** were connected based on the selective inept (SINEPT) data, where the irradiation of NH signals at  $\delta$  7.72 and  $\delta$  8.10 enhanced the carbonyl signals at  $\delta$  162.6 and  $\delta$  163.1, respectively. The presence of one phenolic OH signal in the  $^1\text{H}$ -nmr spectrum of **1**, along with the highly polar nature of the compound, led us to predict the presence of a sulfate group. Attempts at methylation and acetylation led to mixtures of compounds due to partial hydrolysis of the sulfate. Therefore the compound was hydrolyzed under mild conditions to yield compound **2**, the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr chemical shifts of which were identical to the recently published bastadin 13 (9). This led us to place the sulfate on C-34 and to connect C-14/C-9 and C-29/C-33 via ether linkages. It is interesting to note the  $^{13}\text{C}$  chemical shift difference of **1** and **2**, where the carbon resonances observed for C-33, C-35, and C-37 shift to high field and C-34 shifts to low field upon conversion of the sulfated compound to

its phenolic form, leaving the other carbon resonances unchanged. This effect can be easily observed in some recent examples (12,13), where the carbon directly attached to the phenolic group shifts to low field, and the two ortho and the para carbons shift to high field, when going from the acetate to phenol. The presence of the sulfate group in natural products is common, especially in sterols (14). It has in rare instances been observed in some terpenes (15). Compound **1** weakly inhibited binding of [ $^{125}\text{I}$ ]ET-1 to the ET<sub>A</sub> receptor in porcine thoracic aorta with an IC<sub>50</sub> value of  $39 \pm 3 \mu\text{M}$  ( $n=3$ ; mean  $\pm$  SEM). Compound **1** only inhibited binding of [ $^{125}\text{I}$ ]ET-3 to the ET<sub>B</sub> receptor in rat cerebellum by  $27 \pm 4\%$  at a concentration of  $70 \mu\text{M}$  and was therefore inactive. Compound **2** inhibited the binding of [ $^{125}\text{I}$ ]ET-1 and [ $^{125}\text{I}$ ]ET-3 by  $32 \pm 6$  and  $30 \pm 4\%$  respectively, at a concentration of  $70 \mu\text{M}$ , and was therefore considered inactive at both ET<sub>A</sub> and ET<sub>B</sub> receptors. In contrast to the

weak inhibition of endothelin binding, the sulfated compound **1** showed no activity against *Bacillus subtilis* at 50  $\mu\text{g}/\text{ml}$ , while compound **2** showed a minimum inhibitory concentration of 6  $\mu\text{g}/\text{ml}$ , suggesting that the free phenolic group at C-34 is required for the antimicrobial activity.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—The 1D nmr spectra were recorded on a Bruker instrument operating at 360 MHz  $^1\text{H}$  and 90.5 MHz for  $^{13}\text{C}$ . The 2D nmr spectra were recorded on a Bruker instrument operating at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ .

**CLASSIFICATION OF THE SPONGE.**—The sponge *Iantibella* sp. was collected using scuba from a sandy, rubble reef slope, at a depth of 9 m, in the far northern region of the Great Barrier Reef, Queensland, Australia, on 19 April 1987. The sponge was irregularly fan-shaped, with a markedly conulose surface. The color was greenish yellow in life and dark reddish purple in EtOH preservative. The sample is a new species of *Iantibella* (Demospongiae, Verongida, Ianthellidae) as described by Kelly-Borges and Bergquist (manuscript in preparation). The skeleton of this new species of *Iantibella* is a simple two-dimensional reticulation of anastomosing fibers, with dendritic spikes extending outwards from the plane of the sponge. A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No 003:00604).

**EXTRACTION AND ISOLATION OF 1.**—*Iantibella* sp. (54 g wet wt) was extracted and blended with EtOH (1 liter). The EtOH extract was concentrated to dryness (2.44 g) and partitioned between *n*-BuOH and  $\text{H}_2\text{O}$ . The *n*-BuOH partition was concentrated to dryness (1.18 g) and chromatographed on a LH-20 column with MeOH. A portion (25 mg) of the fraction containing compound **1** (54.5 mg) was further purified on a Si gel column with step gradients of EtOAc, EtOAc-MeOH (9:1), and MeOH. A fraction eluted with EtOAc-MeOH (9:1) contained compound **1** (16.5 mg, 0.06% wet wt): uv (MeOH)  $\lambda$  max 280 nm ( $\epsilon$  4800), 204 ( $\epsilon$  74,000);  $^1\text{H}$  and  $^{13}\text{C}$  nmr see Table 1; hrfabms  $m/z$  [ $\text{M}-\text{Na}$ ] $^-$  1018.8064 [ $\text{C}_{34}\text{H}_{27}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_4\text{O}_{11}\text{S}$  required 1018.8090 ( $\Delta$  2.6 mmu)].

**PREPARATION OF 2.**—34-Sulfatobastadin 13 (31 mg) was hydrolyzed with 2 N HCl (250  $\mu\text{l}$ ) in MeOH (500  $\mu\text{l}$ ) at 50° for 15 min. The solution was diluted with  $\text{H}_2\text{O}$  (2 ml) and partitioned five times with EtOAc (1 ml). The EtOAc

layers were combined and concentrated to dryness (14.4 mg). The semi-pure compound was further purified by hplc on an Altex ODS column (5 $\mu$ , 10mm $\times$ 25 cm) with MeOH- $\text{H}_2\text{O}$  (75:25) to yield bastadin 13 [**2**] (7.9 mg, 28%): uv (MeOH) 280 nm ( $\epsilon$  11,200), 204 ( $\epsilon$  141,000);  $^1\text{H}$  and  $^{13}\text{C}$  nmr see Table 1; hrfabms  $m/z$  [ $\text{M}+\text{H}$ ] $^+$  940.8694 [ $\text{C}_{34}\text{H}_{29}^{79}\text{Br}_2^{81}\text{Br}_2\text{N}_4\text{O}_8$  required 940.8681 ( $\Delta$  1.3 mmu)].

**ENDOTHELIN BINDING ASSAY METHODS.**—Porcine thoracic aortas, obtained through a local abattoir, were immediately stripped and cleaned of blood vessels and fat, and suspended in 10 volumes of 10 mM Tris, pH 7.4, containing 250 mM sucrose. The tissue was homogenized 5 times using a Polytron for 30 sec at setting 6. The mixture was centrifuged at 1000 $\times g$  for 15 min, and the resulting supernatant was filtered through 70 mesh nylon gauze. The suspension was then centrifuged at 48,000 $\times g$  for 30 min, and the resulting pellets were washed twice more. The pellets were stored at -70°.

Cerebella were removed from Sprague-Dawley rats [TAC:N(SD)fBR, Taconic Farm, Germantown, NY, 250–400 g] and suspended in 20 volumes of cold Hanks Balanced Salt Solution (HBSS). The cerebellum was homogenized using a Polytron for 20 sec at setting 6. The homogenate was centrifuged for 12 min at 48,000 $\times g$ . The pellet was washed twice with HBSS, and the resulting pellet was resuspended in 15 ml of HBSS and frozen at -70°.

On the day of the experiment, membrane preparations (800  $\mu\text{g}/\text{ml}$  wet for cerebellum, 60 mg/ml wet wt for aorta) were thawed and suspended in 20 mM Hepes buffer, pH 7.4, containing 145 mM NaCl, 5 mM KCl, 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 0.1% bovine serum albumin, and 0.02% bacitracin. Binding experiments were performed by incubating 100  $\mu\text{l}$  of the suspension, 50  $\mu\text{l}$  of either 40 pM [ $^{125}\text{I}$ ]ET-1 or [ $^{125}\text{I}$ ]ET-3 (NEN-DuPont, Boston, MA; specific activity 2200 Ci/mmol), and 50  $\mu\text{l}$  of drug or buffer at a final volume of 0.5 ml for 2 h at 37°. Non-specific binding was determined in the presence of 100 nM unlabeled ET-3 or ET-1. Bound and free radioactivity were separated by vacuum filtration through Whatman GF/C glass fiber filters presoaked in 0.2% powdered milk. Filters were washed three times with 4 ml of ice-cold buffer and counted in a Genesys gamma counter (Schaumburg, IL).

**BACILLUS SUBTILIS MIC.**—Two-fold dilutions of the test compound were prepared in Mueller-Hinton broth supplemented with  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  (16), giving a total volume of 40  $\mu\text{l}$  in the wells of a 96-well plate. Wells were inoculated with *B. subtilis* ATCC strain 6633 bringing the total volume to 50  $\mu\text{l}$  and the cell density to  $10^3\cdot\text{ml}^{-1}$ . Plates were incubated at 37° for 18–24

TABLE 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr Data in  $\text{DMSO}-d_6$ ,<sup>a</sup>

Position	Compound			
	1		2	
	$\delta\text{H}$	$\delta\text{C}$	$\delta\text{H}$	$\delta\text{C}$
1	3.41 (s)	27.9	3.39 (s)	27.7
2	—	150.7	—	151.1
3	—	162.6	—	162.7
4	7.72 (t, 5.8)	—	7.74 (t, 6.0)	—
5	3.22 (dt, 5.8, 7.0)	40.3	3.20 (dt, 7.0, 6.0)	40.4
6	2.54 (t, 7.0)	33.8	2.53 (t, 7.0)	33.82
7	—	130.7	—	130.63
8	6.50 (d, 2.0)	120.1	6.52 (d, 2.0)	120.2
9	—	143.0	—	143.0
10	—	146.7	—	146.8
11	6.85 (d, 8.5)	117.0	6.86 (d, 8.2)	117.1
12	6.81 (dd, 2.0, 8.5)	125.0	6.80 (dd, 2.0, 8.2)	125.0
13	—	—	—	—
14	—	152.3	—	152.3
15	—	111.9	—	111.8
16	7.47 (d, 2.0)	133.1	7.48 (d, 2.0)	133.1
17	—	135.4	—	135.3
18	7.00 (dd, 2.0, 8.3)	129.3	7.01 (dd, 2.0, 8.4)	129.3
19	6.57 (d, 8.3)	117.9	6.58 (d, 8.4)	117.7
20	2.66 (t, 6.5)	33.9	2.68 (t, 6.5)	33.8
21	3.37 (dt, 6.5, 6.3)	39.8	3.37 (dt, 6.0, 6.5)	39.8
22	8.10 (t, 6.3)	—	8.10 (t, 6.0)	—
23	—	163.1	—	163.1
24	—	151.3	—	151.2
25	3.69 (s)	28.4	3.70 (s)	28.4
26	—	137.4	—	137.9
27	7.42 (s)	133.1	7.45 (s)	133.1
28	—	117.2	—	117.1
29	—	146.6	—	146.2
30	—	117.2	—	117.1
31	7.42 (s)	133.1	7.45 (s)	133.1
32	—	—	—	—
33	—	150.3	—	144.5
34	—	138.6	—	141.8
35	—	119.1	—	110.0
36	6.93 (s)	125.9	6.92 (s)	126.0
37	—	133.6	—	128.3
38	5.98 (s)	113.5	6.01 (s)	113.1
$\text{N}^2\text{-OH}$	11.68	—	11.62	—
$\text{N}^{24}\text{-OH}$	11.88	—	11.89	—
34-OH	—	—	9.89	—
10-OH	9.35	—	9.33	—

<sup>a</sup>Table entries are chemical shifts in ppm (multiplicity,  $J$  in Hz) at 360 MHz for  $^1\text{H}$  and 90.5 MHz for  $^{13}\text{C}$ .

h. The MIC was determined visually as the lowest concentration of the compound that produced complete inhibition of growth.

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