Bastadin 21, a Novel Isobastarane Metabolite from the Great Barrier Reef Marine Sponge *Ianthella quadrangulata*

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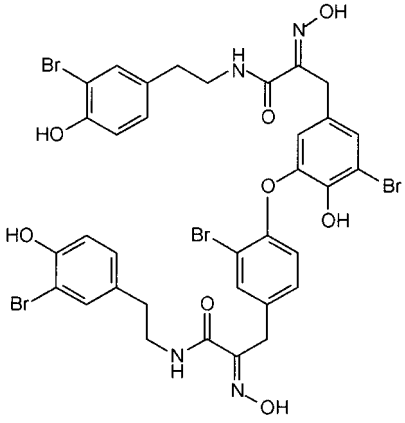
Bastadin 21, a novel tribrominated bastadin with the uncommon isobastarane skeleton, was isolated from the Great Barrier Reef marine sponge *Ianthella quadrangulata*. The structure was elucidated on the basis of the 1D and 2D NMR and MS data of bastadin 21 and its tetramethyl ether.

Sponges of the order Verongida normally afford halogenated tyrosine metabolites,[1] including the bastadin group. To date the isolation of 20 bastadins has been described in the literature.[2] These compounds are derived biosynthetically by the oxidative phenolic coupling of two tyramine-tyrosine units.[3] Bastadins 1−3 are "acyclic", e.g., **1**,[4] and bastadins 4−20 are macrocyclic. The structures of bastadins 13 (**2**), 19, and 20 are based on the isobastarane



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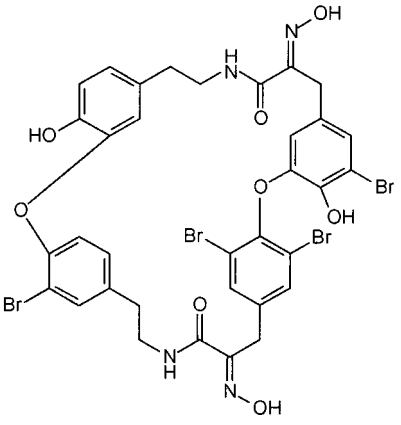

2

skeleton,[2,5,6] but the other macrocyclic bastadins reported to date possess the bastarane skeleton (e.g., **3**, bastadin 18).[6]

A vase-shaped yellow sponge (maroon after storage at −20 °C) was collected from Sykes Reef in the Capricorn Bunker Group and identified as *Ianthella quadrangulata* Bergquist and Kelly-Borges. The EtOH extract of the sponge was concentrated under vacuum and rapidly chromatographed on SiO_2 using petroleum ether to CH_3OH gradients. The fractions eluted using petroleum ether and EtOAc solvent mixtures were found to contain aromatic compounds that were probably bastadins, as well as fats and other lipids. These fractions were combined and rechromatographed on SiO_2 using petroleum ether and EtOAc mixtures. The presumed bastadins were eluted without separation from this second column with solvent mixtures that ranged from 50% EtOAc in petroleum ether to neat EtOAc. The bastadin mixture was then rechromatographed on a reversed-phase (C_{18}) HPLC column using 40% aqueous CH_3OH. Nine fractions were collected. The first contained the major bastadin, which was pure by ^{1}H NMR and appeared to be a novel compound (**4**).

The 400 MHz ^{1}H NMR spectral data of **4** contained six exchangeable resonances: two oxime OH singlets (δ 11.87 and 11.77); two phenolic OH singlets (δ 9.89 and 9.41); and two broad triplets (δ 7.92 and 8.09) that were presumably hydrogens bonded to nitrogen. There were also six upfield resonances, each of which integrated for two protons: δ 3.79 and 3.58 (both broad singlets); δ 3.50, 3.31, 2.79, and 2.61 (all 2H multiplets); and 11 aromatic proton resonances in the ^{1}H NMR spectral data of **4**. The J_{MOD} NMR spectrum contained signals for 34 carbon atoms, including two signals at low field (δ 162.8 and 163.3) that were presumably amide carbonyl carbons, as well as six methylene carbon resonances: δ 27.8, 28.4, 33.6, 33.9, 39.5, and 39.7.

An analysis of the aromatic proton signals in the ^{1}H NMR spectral data of **4**, together with the COSY NMR data and coupling constants, suggested there were three 1,2,4-trisubstituted and one 1,2,3,5-tetrasubstituted aromatic rings in the structure of **4**. The deshielding of two of the aromatic proton resonances (δ 7.58 and 7.53) suggested that they were *ortho* to a bromine atom. The presence of three shielded quaternary aromatic carbons in the ^{13}C NMR spectral data of **4** (δ 110.6, 111.9, and 113.3) suggested a third bromine atom was also incorporated in the structure of **4**; this was confirmed by the presence of a 1:3:3:1 quartet in the LRESIMS of **4** at *m*/*z* 881/883/885/887. Accurate mass measurement of the ions of this quartet suggested that this cluster of ions was the sodium adduct of a bastadin with the molecular formula $C_{34}H_{29}N_4O_8Br_3$. No tribrominated bastadin has been reported previously; indeed, the formula itself is novel.

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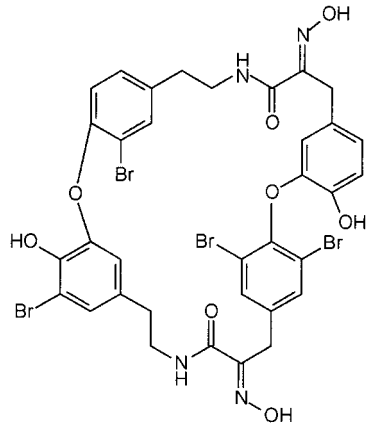

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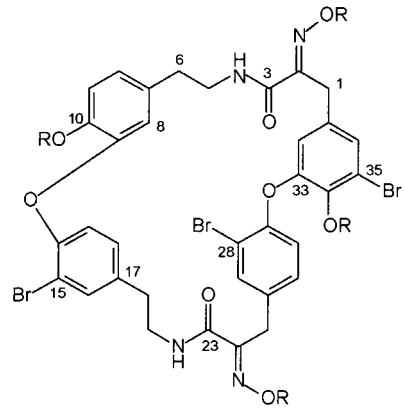
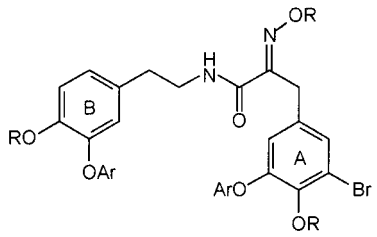


4 R = H
6 R = CH_3

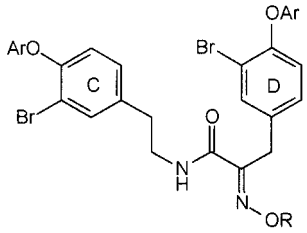


5a R = H
7a R = Me

There were 21 degrees of unsaturation in the structure of **4**, 20 of which were satisfied by the presence of four phenyl rings and two carbonyl and oxime functionalities. This left one degree of unsaturation as yet unaccounted for and suggested that **4** was probably a macrocyclic bastadin.

The 2D NMR spectral data of **4** were used to develop two partial structures (**5a** and **5b**). In addition to the aromatic protons, the COSY spectrum also identified two CH_2CH_2NH spin systems. HMQC and HMBC data were then used to position each spin system, HMBC couplings from the four benzylic methylene and the two phenolic resonances to aromatic carbons being particularly important. For example, the phenolic proton at δ 9.41 (on C-10) showed HMBC coupling to a protonated aromatic carbon at δ 116.9 (C-11) and to two oxygenated carbons at δ 142.9 (C-9) and 146.7 (C-10). The δ 116.9 carbon was determined to be adjacent to the protonated carbon at δ 124.9 (C-12) by *ortho* coupling ($J = 8$ Hz) between their attached protons (δ 6.96 and 6.89, respectively). HMBC correlation of C-12 to the H_2-6 benzylic methylene protons therefore placed the C-10 phenol group *para* to the benzylic methylene, and the adjacent macrocyclic ether linkage at C-9 *meta*. Similarly the C-34 phenol group (δ 9.89) was determined as adjacent to a second macrocyclic ether linkage and *para* to C-1. HMBC correlations from the C-3 amide carbon (δ 162.8) to H_2-5 (δ 3.31) and H_2-1 enabled determination of partial structure **5a**, which contained both phenolic groups. Complete 1D and 2D spectral data and assignments of **4** are provided in Table 1 of the Supporting Information.

As both macrocyclic ether linkages from **5a** are *meta* to their respective benzylic methylenes, a bastarane skeleton is precluded for **4**. Of the two possible ways of combining **5a** and **5b**, bonds may exist between rings B and C and rings A and D, respectively, giving the isobastarane skeleton, or between rings A and C and rings B and D, respectively, yielding an (as yet) undescribed bastarane skeleton. These two isomeric structures cannot be differentiated by normal two- and three-bond HMBC correlations, and NOESY correlations in **4** were ambiguous. Therefore in an attempt to provide conclusive structural data, the tetramethyl ether derivative (**6**) was prepared using MeI and K_2CO_3.[8] The molecular formula of **6**, $C_{38}H_{37}N_4O_8Br_3$, was confirmed by accurate mass measurement. The COSY, HMQC, and HMBC data of **6** (Table 2 of Supporting Information) were used, together with an analysis of the magnitude of the coupling constants in the ^{1}H NMR spectral data, to construct two substructures (**7a** and **7b**) that were analogous to **5a** and **5b**.



5b R = H
7b R = Me

Fragmentation of the molecular ion of bastadin permethyl ethers results in dual cleavage on the nitrogen or carbonyl side of both amide groups; this is also known as "eastern-" and "western-hemisphere" fragmentation,[2] terminology that is based on the traditional manner in which these compounds are drawn (Figure 1). The LREIMS of **6** gave rise to two isotopic ion clusters (*m/z* 330/332, a 1:1 doublet, and *m/z* 434/436/438, a 1:2:1 triplet) that have the formulas $[C_{17}H_{17}O_2Br]^{\bullet+}$ and $[C_{17}H_{12}N_2O_2Br_2]^{\bullet+}$, respectively. These fragment ions are consistent only with an isobastarane skeleton for **4** and **6**.

It was possible to assign the stereochemistry of the oxime groups in **4** using ^{13}C NMR chemical shifts. Since the α-benzylic carbons resonated at δ 27.8 and 28.4 in **4**, both oxime groups must have *E* geometry.[7]

The remaining *I. quadrangulata* fractions contained complex mixtures with up to 20 bastadin-like compounds in total. ^{1}H NMR data indicated compounds similar to other known bastadins, but none were isolated in sufficient quantity and purity to enable full structural elucidation.

Experimental Section

General Experimental Procedures. Normal-phase TLC was performed on Merck DC-Alufolien Kieselgel 60 F_{254} sheets. Reversed-phase TLC was performed on glass sheets coated with Merck Octadecyl modified silica gel (UV_{254}). The TLC plates were visualized at both short (254 nm) and long (366 nm) wavelengths and then either placed in an atmosphere of

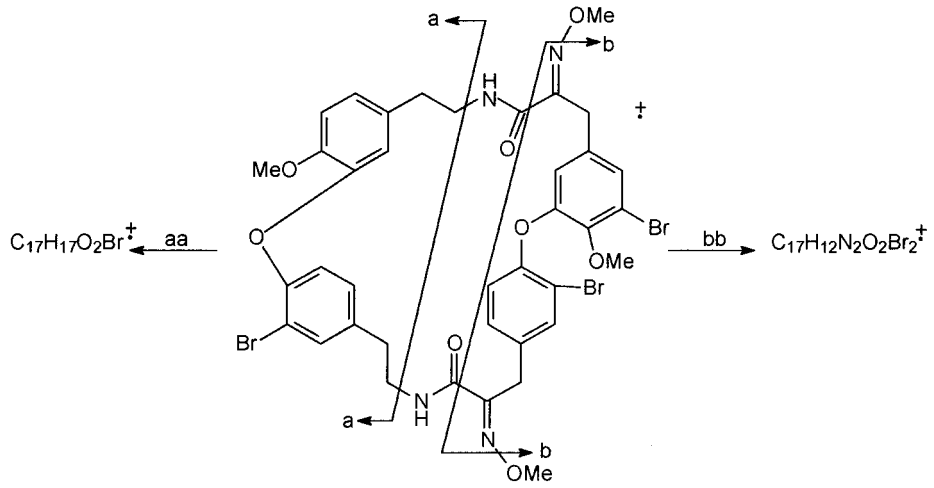


Figure 1. The ions formed as a consequence of the two possible fragmentation pathways of the molecular ion of **6**.

I_2 or sprayed with a 1% solution of vanillin in H_2SO_4. Crude extracts and other impure mixtures were subjected to vacuum-assisted liquid chromatography[9] on Merck Kieselgel 60PF (TLC) SiO_2. Analytical scale high-performance liquid chromatography (HPLC) was performed using a NovaPak C_{18} column (60 Å, 4 μm, 3.9 × 150 mm). Preparative scale HPLC was performed using a NovaPak C_{18} RCM column (60 Å, 6 μm, 25 × 100 mm), and compounds were detected using a Gilson 132 refractive index detector. Ultraviolet spectra were recorded on a Varian Cary 4E spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR as KBr disks. All NMR spectral data were recorded on an Avance spectrometer at 400 and 100 MHz, respectively, using standard parameter sets, and calibrated using the respective residual solvent peaks: DMSO-d_6, δ 2.60 and 39.5 ppm; $CDCl_3$, δ 7.26 and 77.0 ppm.

Animal Material. A specimen of *Ianthella quadrangulata* Bergquist and Kelly-Borges, 1995 was collected by scuba diving, under the authority of the GBRMPA Collection Permit G92-524, in about 10 m of water from Sykes Reef in the Capricorn Bunker Group, Great Barrier Reef, and identified by Stephen Cook, Queensland Museum, Brisbane, Australia.

Extraction and Isolation. A frozen sample (45 g) of the sponge was extracted exhaustively with EtOH to afford the crude extract (6 g). SiO_2 (4 g) was added to an EtOH solution (25 mL) of the extract and dried under vacuum. This loaded SiO_2 was placed on the top of a SiO_2 column and chromatographed using solvent gradients from petroleum ether (40−60) to CH_3OH. The material eluted with petroleum ether−EtOAc gradients was combined and rechromatographed on SiO_2 using petroleum ether−EtOAc gradients. The fractions eluted with petroleum ether−EtOAc (1:1) to EtOAc gradients (0.128 g) were combined and purified on a preparative reversed-phase (C_{18}) HPLC column [CH_3OH-H_2O (60:40)], flow rate 8 mL/min, t_R **4**, 17.2 min (17 mg, 0.1% by dry weight).

Bastadin-21 (4): amorphous white solid; UV (CH_3OH) λ_{max} (log ϵ) 195 (4.56), 279 (3.40), 384 (2.99) nm; IR (KBr) ν_{max} 3422, 2925, 1743, 1654, 1697, 1490, 1380, 1235, 1044 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 2.61 (2H, m, H-6), 2.79 (2H, m, H-20), 3.31 (2H, m, H-5), 3.50 (2H, m, H-21), 3.58 (2H, br s, H-1), 3.79 (2H, br s, H-25), 6.44 (1H, d, J = 2 Hz, H-38), 6.62 (1H, d, J = 2 Hz, H-8), 6.69 (1H, d, J = 8 Hz, H-19), 6.89 (1H, dd, J = 8, 2 Hz, H-12), 6.92 (1H, d, J = 8 Hz, H-28), 6.96 (1H, d, J = 8 Hz, H-11), 7.10 (1H, dd, J = 8, 2 Hz, H-18), 7.14 (1H, d, J = 2 Hz, H-36), 7.17 (1H, dd, J = 8, 2 Hz, H-27), 7.53 (1H, d, J = 2 Hz, H-31), 7.58 (1H, d, J = 2 Hz, H-16), 7.92 (1H, br t, 4-N*H*), 8.09 (1H, br t, 22-N*H*), 9.41 (1H, s, 10-O*H*), 9.89 (1H, s, 34-O*H*), 11.77 (1H, s, 2-O*H*), 11.87 (1H, s, 24-O*H*); ^{13}C NMR (100 MHz, DMSO-d_6) 27.8 (C-1), 28.4 (C-25), 33.6 (C-20), 33.9 (C-6), 39.5 (C-21), 39.7 (C-5), 110.6 (C-35), 111.9 (C-15), 113.3 (C-30), 116.9 (C-11), 117.2 (C-38), 117.8 (C-19), 120.0 (C-8), 120.2 (C-28), 124.9 (C-12), 127.2 (C-36), 128.9 (C-37), 129.2 (C-18), 129.5 (C-27), 130.6 (C-7), 133.1 (C-16), 133.4 (C-31), 134.5 (C-26), 135.5 (C-17), 142.9 (C-9), 143.4 (C-34), 145.1 (C-33), 146.7 (C-10), 150.9 (C-29), 151.4 (C-2), 151.7 (C-24), 152.2 (C-14), 162.8 (C-3), 163.3 (C-23); ESIMS m/z [M + Na]$^+$ 887 (40), 885 (100), 883 (96), 881 (32); HRESIMS m/z 886.9394 (calcd for $C_{34}H_{29}N_4O_8{}^{81}Br_3Na$, 886.9391), 884.9406 (calcd for $C_{34}H_{29}N_4O_8{}^{81}Br_2{}^{79}BrNa$, 884.9399), 882.9412 (calcd for $C_{34}H_{29}N_4O_8{}^{81}Br{}^{79}Br_2Na$, 882.9415), 880.9431 (calcd for $C_{34}H_{29}N_4O_8{}^{79}Br_3Na$, 880.9433).

Preparation of the Tetramethyl Ether (6) of Bastadin-21 (4). K_2CO_3 (0.4 g) and MeI (0.6 mL) were added to an anhydrous acetone solution (15 mL) of **4** (17 mg, 0.02 mmol) and stirred under an atmosphere of N_2 for 17 h. The reaction mixture was concentrated under vacuum, resuspended in EtOAc, and filtered to remove K_2CO_3. The K_2CO_3 was then washed with EtOAc, and the reaction mixture was chromatographed on SiO_2 using petroleum ether−EtOAc mixtures to afford **6** (12 mg, 0.013 mmol, 65% yield) as a pale yellow gum: UV (EtOH) λ_{max} (log ϵ) 205 (4.71) nm; IR (KBr) ν_{max} 3045, 2970, 2954, 2854, 1735, 1730, 1676, 1654, 1376, 1285, 1125, 1076, 1045, 744 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) 2.72 (2H, t, J = 6 Hz, H-6), 2.78 (2H, t, J = 6 Hz, H-20), 3.47 (2H, q, J = 6 Hz, H-5), 3.58 (2H, q, J = 6 Hz, H-21), 3.62 (2H, br s, H-1), 3.65 (3H, s, 2N-O*Me*), 3.83 (3H, s, 10-O*Me*), 3.87 (2H, br s, H-25), 3.92 (3H, s, 34-O*Me*), 3.99 (3H, s, 24N-O*Me*), 6.48 (1H, d, J = 2 Hz, H-38), 6.62, (1H, br t, 4-N*H*), 6.66 (1H, d, J = 8 Hz, H-19), 6.67 (1H, br t, 22-N*H*), 6.69 (1H, d, J = 2 Hz, H-8), 6.77 (1H, d, J = 8 Hz, H-28), 6.93 (1H, d, J = 8 Hz, H-11), 6.93 (1H, dd, J = 2, 8 Hz, H-12), 6.94 (1H, dd, J = 2, 8 Hz, H-18), 7.11 (1H, dd, J = 2, 8 Hz, H-27), 7.21 (1H, d, J = 2 Hz, H-36), 7.43 (1H, d, J = 2 Hz, H-16), 7.54 (1H, d, J = 2 Hz, H-31); ^{13}C NMR ($CDCl_3$ 100 MHz) 28.7 (C-25), 29.1 (C-1), 34.5 (C-20), 34.6 (C-6), 40.5 (C-21), 40.9 (C-5), 56.3 (*C*H$_3$O-10), 61.0 (*C*H$_3$O-34), 62.8 (*C*H$_3$O-2[a]), 63.0 (*C*H$_3$O-24[a]), 113.0 (C-15), 113.5 (C-12), 114.2 (C-30), 114.5 (C-35), 118.0 (C-19), 119.0 (C-38), 120.9 (C-28), 121.1 (C-8), 125.0 (C-11), 128.3 (C-36), 128.8 (C-18), 129.4 (C-27), 132.0 (C-7), 133.2 (C-37), 133.6 (C-16), 134.5 (C-31), 135.0 (C-17), 135.0 (C-26), 145.0 (C-9), 146.1 (C-34), 149.5 (C-10), 151.0 (C-2), 151.2 (C-29), 151.5 (C-24), 153.2 (C-14), 153.5 (C-33), 162.0 (C-3), 162.5 (C-23) ([a]may be interchanged); EIMS m/z [M]$^+$ 920 (24), 918 (75), 916 (75), 914 (25), 438 (20), 436 (52), 434 (22), 376 (31), 374 (28), 332 (100), 330 (91); HRESIMS m/z [M + Na]$^+$ 943.0012 (calcd for $C_{38}H_{37}N_4O_8{}^{81}Br_3Na$, 942.9998), 941.0027 (calcd for $C_{38}H_{37}N_4O_8{}^{81}Br_2{}^{79}BrNa$, 941.0027), 939.0032 (calcd for $C_{38}H_{37}N_4O_8{}^{81}Br{}^{79}Br_2Na$, 939.0042), 937.0054 (calcd for $C_{38}H_{37}N_4O_8{}^{79}Br_3Na$, 937.0059).

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Supporting Information Available: Tables showing the ^{1}H and ^{13}C 1D and 2D NMR data of **4** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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