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Bistellettazines A−C and Bistellettazole A: New Terpenyl−Pyrrolizidine and Terpenyl−Imidazole Alkaloids from a Southern Australian Marine Sponge, *Stelletta* sp

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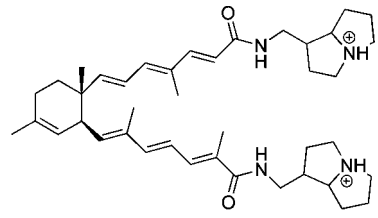
ABSTRACT



bistellettazine A

Four new alkaloids have been isolated during chemical investigations into a southern Australian marine sponge, *Stelletta* sp. Detailed spectroscopic analysis, supported by chemical degradation and partial synthesis, permitted structure elucidation of bistellettazines A−C (1−3), the first reported examples of terpenyl-pyrrolizidine conjugates, and bistellettazole A (4), a unique cyclic terpenyl-imidazole conjugate. The alkaloids 1−4 feature unprecedented carbon skeletons that are proposed to share a common convergent biosynthetic origin, arising via the biogenic equivalent of a Diels−Alder addition between two hypothetical polyenyl norsesquiterpene precursors.

In our search for potential anticancer compounds from marine invertebrates, we examined a library of >2500 marine sponges obtained over a period of 20+ years from locations along the coasts of southern Australia and Antarctica. Solvent extracts prepared from these samples were screened for cytotoxic activity against a panel of human cancer cell lines, leading to the prioritization of >100 samples. Among the prioritized samples was a *Stelletta* sp. obtained in 1995 during scientific trawling operations (epibenthic sled) at a depth of 90 m in the Great Australian Bight.

Sequential hexane, DCM, MeOH, and H_2O trituration of an aqueous EtOH extract of a *Stelletta* specimen (CMB-01936) followed by reversed-phase HPLC returned four novel alkaloids identified as bistellettazines A−C (**1**−**3**) and bistellettazole A (**4**), the first reported examples of terpenyl−pyrrolizidine and cyclic terpenyl−imidazole conjugates, respectively (Chart 1). The structure elucidation of **1**−**4**, based on detailed spectroscopic analysis, chemical degradation, and partial synthesis, is presented below, along with a proposal for a common biosynthetic pathway involving two hypothetical polyenyl norterpene precursors and the biogenic equivalent of a Diels−Alder addition.

Bistellettazine A (**1**) displayed a +ve ESI mass spectrum dominated by a doubly charged molecular ion (M^{2+}, *m*/*z* 321) and a singly charged fragment ion ($M - H^+$, *m*/*z* 641). Analysis of high-resolution mass measurement for the $M - H^+$ fragment ion suggested a molecular formula ($C_{41}H_{62}N_4O_2$, −0.1 Δmmu) incorporating two +ve charged functional groups and 14 double-bond equivalents (DBE). The NMR (DMSO-d_6) data for **1** (Table S1b, Supporting Information)

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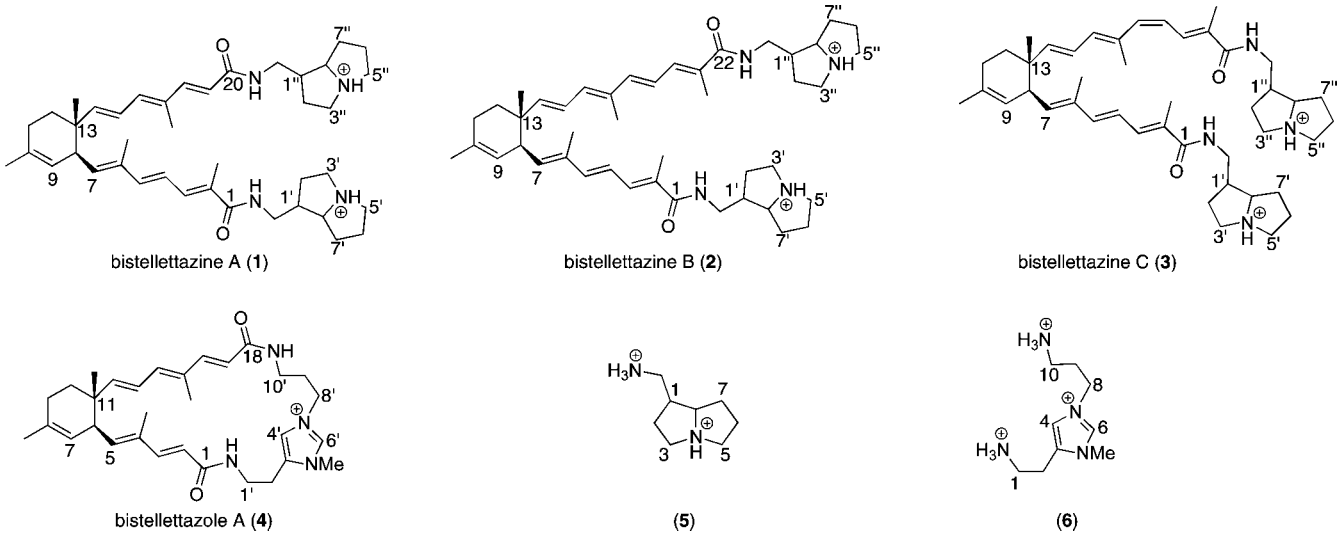
Chart 1



revealed two exchangeable amide protons (δ_H 8.26 (1-CON*H*, t, 5.9 Hz) and 8.05 (20-CON*H*, t, 5.9 Hz)) with HMBC correlations to two amide carbonyl carbons (δ_C 168.5 (C-1) and 165.8 (C-20), respectively) and two amino methylene carbons (δ_C 40.1 (1′-*C*H$_2$, 39.7 (1″-*C*H$_2$). The NMR (MeOH-d_4) data for **1** (Table S1a, Supporting Information), which provided better dispersion compared to NMR (DMSO-d_6) data, further confirmed the presence of two secondary amides and revealed 14 sp^2 olefinic carbons accounting for 9 DBE and requiring that **1** be pentacyclic. Further examination of the NMR (MeOH-d_4) data revealed a sequence of 2D COSY and HMBC correlations characteristic of the acyclic chain from the C-1 amide carbonyl to C-7. An *E* stereochemistry was assigned about all three double bonds Δ^2, Δ^4, and Δ^6 on the basis of $J_{4,5}$ 15.1 Hz and diagnostic ROESY correlations between H-4 and 2-Me and H-7 and H-5. A comparable sequence of 2D COSY and HMBC correlations characterized the acyclic chain from C-14 to the C-20 amide carbonyl. Again, an *E* stereochemistry was assigned about all three double bonds Δ^{14}, Δ^{16}, and Δ^{18} on the basis of $J_{14,15}$ 15.1 Hz and $J_{18,19}$ 15.4 Hz and diagnostic ROESY correlations between H-14 and H-16, H-15 and 17-Me, H-16 and H-18, and H-19 and 17-Me. A doublet multiplicity together with HMBC correlations to the sole sp^3 quaternary carbon (C-13, δ_C 39.8 (s)), bearing a tertiary methyl (13-Me, δ_H 0.98 (s)), positioned H-14 adjacent to C-13. A sequence of COSY and HMBC correlations extended the connectivity from C-13 to C-7, while the need to accommodate a quaternary C-13 and tertiary C-8 was strongly suggestive of a C-8 to C-13 ring closure. This latter ring formation was confirmed by HMBC correlations from H-7 and H-9 to C-13, as well as ROESY correlations between H-14 and H-8. This latter observation, together with a ROESY correlation between 13-Me and H-7 (Figure S1d, Supporting Information), also established the relative stereochemistry about the cyclohexene ring system as indicated. The arguments presented above accommodate the structure fragment from the C-1 to C-20 secondary amides, incorporating all available sp^2 carbons, and extending to include 1′/1″-CH$_2$ residues. These assignments account for all but 4 DBE and the elements of $C_{14}H_{26}N_2^{2+}$.

Bistellettazine A (**1**) would not form acetate or BOC derivatives under standard experimental conditions, strongly suggesting that the remaining heteroatoms were incorporated into two tertiary ammonium moieties. This interpretation was further supported by the observation of six deshielded resonances in the remaining ^{13}C NMR (MeOH-d_4) data for **1**, consistent with four *N*-methylenes (δ_C 55.62/55.64 and 55.98/56.01) and two *N*-methines (δ_C 72.42/72.47)—as might be expected for twin pyrrolizidine functionalities. The broad, heavily coupled, and overlapping nature of the pyrrolizidine ^{1}H NMR (MeOH-d_4) resonances in **1** prevented an unambiguous structure assignment for these alkaloid residues. In an attempt to overcome this limitation, we undertook a chemical degradation of **1** (hydrogenation (Pd/C, H$_2$) followed by acid hydrolysis) to yield a single alkaloid product **5**. The high-resolution mass spectral data for **5** revealed a singly charged fragment ion (M $-$ H$^+$) consistent with a molecular formula ($C_8H_{18}N_2$, 0.1 Δmmu) for a diammonium salt with 2 DBE. The absence of sp^2 carbon resonances in the ^{13}C NMR data (Tables S5a and S5b, Supporting Information) confirmed **5** as bicyclic. Analysis of the NMR (MeOH-d_4) data (Table S5a, Supporting Information) revealed a sequence of 2D COSY correlations mapping the carbon skeleton as shown. A comparable analysis of the NMR (DMSO-d_6) data (Table S5b, Supporting Information) further confirmed the carbon skeleton as shown and also revealed correlations from the 4-N*H* (δ_H 11.59) to 3-Ha, 5-Ha, and 8-H, positioning the tertiary amine at the pyrrolizidine bridgehead as indicated. Optical rotation ($[\alpha]_D$ = +10) and a Marfey's analysis (Figure S5b, Supporting Information) confirmed **5** to consist of only one enantiomer. Examination of the 2D ROESY NMR data for **5** revealed a weak correlation between H-8 and 1-C*H*a, but this was

insufficient to assign the relative stereochemistry of the pyrrolizidine. Although **5** is new to natural products chemistry, two stereoisomers were reported in 2006 by Pfizer researchers[1] as synthetic intermediates in a medicinal chemistry investigation of pyrrolizidine amides as 5-HT_4 receptor antagonists and agonists. Unfortunately the spectroscopic characterization reported at that time was inadequate for a definitive comparison, and the lack of optical rotation data prevented a comparative assignment of stereochemistry.

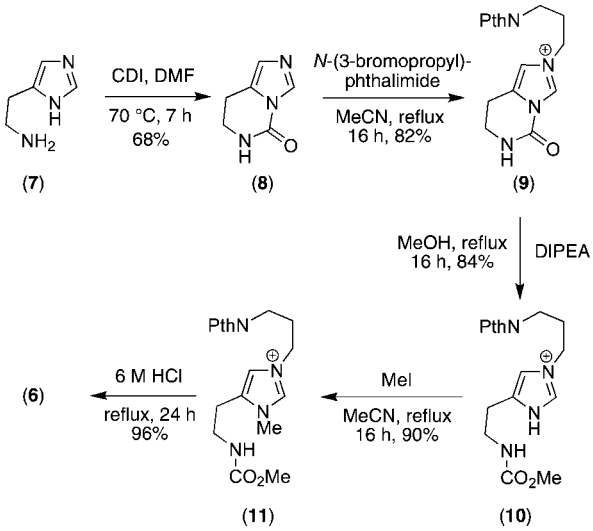
Bistellettazine B (**2**) displayed similar mass spectral properties to **1**, with a +ve ESI mass spectrum dominated by a doubly charged molecular ion (M^{2+}, *m*/*z* 341) and a singly charged fragment ion ($M - H^+$, *m*/*z* 681). High-resolution analysis of the $M - H^+$ fragment ion was consistent with a molecular formula ($C_{44}H_{64}N_4O_2$, 2.0 Δmmu) incorporating two +ve charged functional groups and 15 DBE. Again, in an analysis similar to that presented above for **1**, the NMR (DMSO-d_6) data for **2** (Table S2b, Supporting Information) revealed two exchangeable amide protons (δ_H 8.05/8.07 (1-CON*H*/22-CON*H*, t, 5.8 Hz)) with HMBC correlations to two amide carbonyl carbons (δ_C 168.47/168.49 (C-1 and C-22)) and two equivalent amino methylene carbons (δ_C 40.1 (1′/1″-*C*H_2). The NMR (MeOH-d_4) data for **2** (Table S2a, Supporting Information) provided better dispersion of resonances and revealed 16 sp^2 olefinic carbons accounting for 10 DBE and defining **2** as pentacyclic. These observations, together with significant similarities in the NMR data between **1** and **2**, suggested that **2** was a biosynthetic homologue of **1**, featuring a common C-1 to C-13 terpenyl substructure but differing about the C-13 terpenyl side chain. That this side chain in **2** featured a propenyl moiety inserted adjacent to the terminal amide (C-22) was confirmed by 2D COSY and HMBC (MeOH-d_4) correlations (Table S2a, Supporting Information). Key observations included HMBC correlations from H-20 (δ_H 6.98) to the amide carbonyl (C-22, δ_C 172.6) and COSY correlations between H-20 and H-19 (δ_H 6.53). The all-*E* stereochemistry about Δ^2, Δ^4, Δ^6, Δ^{14}, and Δ^{16} and the relative stereochemistry about C-8 and C-13 in the cyclohexenyl moiety in **2** were determined to be the same as those for **1**, based on the observation of comparable coupling constants and 2D ROESY NMR (MeOH-d_4) correlations. An *E* stereochemistry was also assigned to Δ^{18} on the basis of $J_{18,19}$ 15.0 Hz and to Δ^{20} on the basis of a 2D ROESY NMR (MeOH-d_4) correlation between H-19 and 21-Me. The presence of twin pyrrolizidine amides in **2** (incorporating the amine **5**) was inferred from comparisons of NMR (MeOH-d_4) data with **1** was assigned on spectroscopic and biogenetic grounds by comparison to the cometabolite **1**.

Bistellettazine C (**3**) was determined to be isomeric with **2**, with the two isomers displaying a very high degree of similarity between their spectroscopic data (Tables S3a and S3b, Supporting Information). The only significant spectroscopic difference between **2** and **3** was in the NMR (MeOH-d_4) resonances for H-18 and H-19, which in the latter appeared as an overlapping multiplet (δ_H 6.23) suggestive of a differing Δ^{18} stereochemistry. This hypothesis was confirmed by a diagnostic 2D ROESY NMR correlation between 17-Me and H-20, confirming a *Z* Δ^{18} stereochemistry for **3**. All other stereochemical assignments, and the inclusion of twin pyrrolizidine amides, were assigned on the basis of comparable arguments as presented above for **2**.

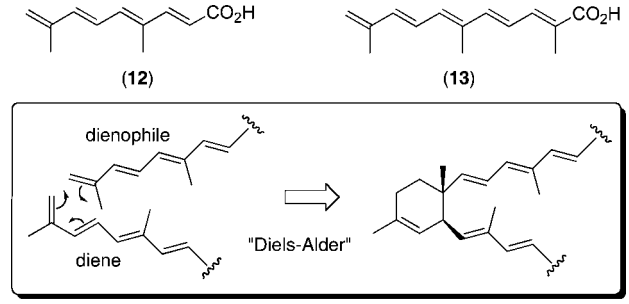
Bistellettazole A (**4**) returned a molecular/pseudomolecular ion in the high-resolution mass spectrum consistent with the formula $C_{31}H_{43}N_4O_2$ (−0.5 Δmmu). Unlike the bistellettazines, the +ve ESI mass spectrum of **4** did not feature doubly charged ions, requiring that $C_{31}H_{43}N_4O_2$ correspond to either the singly charged molecular species (M^+) or a pseudomolecular $M + H^+$ ion. The NMR (DMSO-d_6) data for **4** (Table S4b, Supporting Information) revealed two exchangeable amide protons (δ_H 7.9 (1-CON*H*, t, 5.8 Hz) and 8.0 (18-CON*H*, t, 5.8 Hz)) with HMBC correlations to two amide carbonyl carbons (δ_C 165.6 (C-1) and 166.4 (C-18), respectively) and two amino methylene carbons (δ_C 36.1 (C-1′) and 35.6 (C-10′), respectively). The NMR (MeOH-d_4) data for **4** (Table S4a, Supporting Information) provided better dispersion of resonances compared to DMSO-d_6 and revealed a further 15 sp^2 carbons attributed to a total of seven double bonds and an "imine-like" moiety and suggestive that **4** be tricyclic. Following a similar approach as outlined above for the bistellettazines, analysis of the 2D COSY and HMBC NMR (MeOH-d_4) data revealed correlations consistent with (a) a dienyl amide (C-1 to C-5) attached to (b) a cyclohexenyl moiety (C-6 to C-11), in turn further substituted at C-11 by (c) a tertiary methyl (11-Me) and (d) a trienyl amide (C-12 to C-18)—the (b) to (d) substructural units being in common with **1**. An *E* stereochemistry was assigned to Δ^2 and Δ^4 on the basis of $J_{2,3}$ 15.3 Hz and 2D ROESY NMR (MeOH-d_4) correlations between H-3 and H-5. The relative stereochemistry about the cyclohexenyl ring was established as indicated, by ROESY correlations between H-5 and 11-Me, and H-6 and H-12. An all-*E* stereochemistry was assigned about Δ^{12}, Δ^{14}, and Δ^{16} on the basis of $J_{12,13}$ 14.8 Hz and $J_{16,17}$ 15.3 Hz and the shielded chemical shift for 15-Me (δ_C 12.9), which was in common with the comparable structure fragment (17-Me, δ_C 12.7) in **1** that was independently determined to have an *E* stereochemistry by diagnostic ROESY correlations. Further examination of the 2D COSY and HMBC NMR (MeOH-d_4) data permitted extension of the assigned structure from 1-CON*H* through a 1,2-ethanyl moiety to C-2′, and 18-CON*H* through a 1,3-propanyl moiety to C-8′, with the remaining structure fragment being nominally attributed to an *N*-methylimidazole (C-3′−N-7′). Degradation of **4** as described for **1** returned the imidazole hydrochloride salt **6**. Spectroscopic analysis (Table S6, Supporting Information) supported the identity of **6**, which was confirmed by total synthesis as outlined in Scheme 1, utilizing the method of Cohen.[1] In this synthesis, histamine (**7**) is converted to the cyclic urea **8**, alkylated to **9**, ring opened to **10**, methylated to **11**, and finally deprotected to yield imidazole **6** identical in all respects to that obtained from chemical degradation of **4**. Thus, the total relative stereostructure for bistellettazole A (**4**) can be assigned as shown.

(1) Jain, R.; Cohen, L. A. *Tetrahedron* **1996**, *52*, 5363.

Scheme 1. Synthesis of Hydrolysis Product **6**



Scheme 2. Plausible Biosynthetic Pathway for **1**−**4**



diene (**13**) + dienophile (**12**) + amine (**5**) = (**1**)
diene (**13**) + dienophile (**13**) + amine (**5**) = (**2-3**)
diene (**12**) + dienophile (**12**) + amine (**6**) = (**4**)

While the alkaloids **1**−**4** span three different carbon skeletons, they incorporate numerous structural features in common, including the cyclohexenyl moiety. A possible "common" biosynthetic pathway to these alkaloids could involve the hypothetical norsesquiterpene carboxylic acid precursors **12** and **13**. The biogenic equivalent of a Diels−Alder addition between a dienophile **12** and diene **13** as shown in Scheme 2 could yield the terpenyl carbon skeleton of **1**, while dimerization of **13** could lead to the terpenyl carbon skeleton of **2** and **3**. Likewise, Diels−Alder dimerization of **12** could result in the terpenyl carbon skeleton of **4**. Incorporation of the respective amide moieties could take place prior to or post the biogenic Diels−Alder additions.

The genus *Stelletta* is well-known in the marine natural products literature as a source of unusual terpenes and alkaloids, including terpenyl−indolizadines,[2] terpenyl−guanidines,[3−5] and terpenyl−imidazoles,[6,7] selected examples of which have been described as inhibitors of Ca^{2+}/calmodulin-dependent phosphodiesterase,[5,7] antibacterials,[5−7] antifungals,[2,3,5] metamorphosis inducers in ascidian larval development,[4] activators of RNA cleavage,[3] and cytotoxic agents against K562 epithelium cells.[2]

The bistellettazines A-C (**1**−**3**) and bistellettazole A (**4**) represent a new contribution to our knowledge of *Stelletta* alkaloids, and natural product alkaloids in general, while the proposed biosynthesis suggests a possible route for biomimetic synthesis. Studies directed at the biological properties of **1**−**4** remain a work in progress.

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Supporting Information Available: Includes general experimental methods, sample collection, extraction, fractionation, and characterization of all natural products and synthetics, including tabulated NMR data and ^{1}H NMR spectra.This material is available free of charge via the Internet at http://pubs.acs.org.

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(2) Hirota, H.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1990**, *31*, 4163.
(3) Shin, J.; Seo, Y.; Cho, K. W.; Rho, J. R.; Sim, C. J. *J. Nat. Prod.* **1997**, *60*, 611.
(4) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 5555.
(5) Tsukamoto, S.; Yamashita, T.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1999**, *64*, 3794.
(6) Matsunaga, S.; Yamashita, T.; Tsukamoto, S.; Fusetani, N. *J. Nat. Prod.* **1999**, *62*, 1202.
(7) Tsukamoto, S.; Yamashita, T.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1999**, *40*, 737.