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Latonduines A and B, New Alkaloids Isolated from the Marine Sponge *Stylissa carteri*: Structure Elucidation, Synthesis, and Biogenetic Implications

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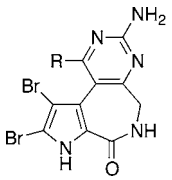
ABSTRACT



Latonduine A R=H
Latonduine B R=CO_2H

Latonduines A (6) and B (7), two new alkaloids with unprecedented heterocyclic skeletons, have been isolated from the Indonesian marine sponge *Stylissa carteri*. The structures of the latonduines were elucidated by analysis of spectroscopic data and confirmed by the total synthesis of latonduine A (6). It is proposed that ornithine is the biogenetic precursor to the aminopyrimidine fragment of the latonduines.

The $C_{11}N_5$ skeleton of oroidin (**1**)[1] represents the formal biogenetic building block for a diverse family of monomeric and dimeric sponge alkaloids[2] that includes dibromophakellin (**2**),[3] hymenialdisine (**3**),[4] and sceptrin (**4**).[5] The "oroidins" hymenialdisine (**3**) and debromohymenialdisine (**5**)[6] have attracted particular attention[7] because of their ability to inhibit protein kinases,[8,9] modulate the proinflammatory transcription factor NF-κB,[10] inhibit the G2 cell-cycle checkpoint,[9] and slow joint and cartilage deterioration associated with osteoarthritis in animal models.[11] As part of an ongoing investigation of bioactive sponge metabolites,[12] we were prompted to investigate an extract of the Indonesian sponge *Stylissa*

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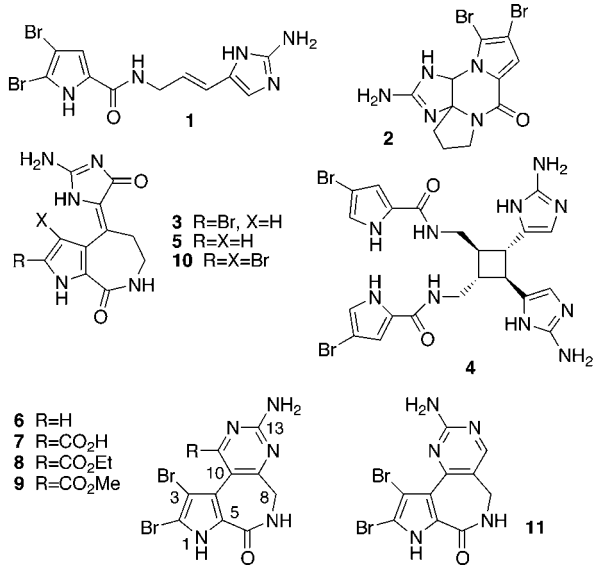
carteri because it showed significant in vitro cytotoxicity against human cancer cell lines. Bioassay-guided fractionation of the extract gave complex cytotoxic mixtures that are still under investigation and an inactive fraction containing the two novel alkaloids latonduines A (**6**) and B (**7**). The structures of latonduines A (**6**) and B (**7**) were elucidated by spectroscopic analysis and confirmed by the total synthesis of latonduine A (**6**) as described below.



Specimens of *S. carteri* (Dendy) (Demospongiae, order Halichondrida, family Dictyonellidae) were collected by hand using SCUBA on shallow reefs off of Latondu Island, Taka Bonerate, Indonesia. Freshly collected sponge (50 g) was preserved on site in EtOH for 2 days at rt after which the EtOH was discarded and the sample was frozen for transport to Vancouver. The frozen sponge was subsequently extracted exhaustively with MeOH. Concentration of the MeOH extract in vacuo gave an aqueous suspension that was partitioned between H_2O and EtOAc. Fractionation of the EtOAc soluble materials by sequential application of Sephadex LH20 chromatography (eluent: 80% MeOH/20% CH_2Cl_2) and reversed-phase HPLC (eluent: 45% MeOH/55% H_2O) gave pure samples of latonduine A (**6**) (2.9 mg), latonduine B ethyl ester (**8**) (2.8 mg), and latonduine B methyl ester (**9**) (0.5 mg) as pale yellow crystalline solids.

Latonduine A (**6**) gave a 1:2:1 M^+ ion cluster at *m*/*z* 371, 373, and 375 in the LREIMS, indicating that the molecule contained two bromine atoms. A HREIMS analysis of **6** showed that the mass of the M^+ cluster peak at *m*/*z* 372.9002 was appropriate for a molecular formula of $C_{10}H_7N_5O^{79}Br^{81}Br$ (calcd 372.8997) requiring seven sites of unsaturation. The ^{13}C NMR spectrum (DMSO-d_6) of **6** contained 10 well-resolved resonances consistent with the HREIMS data, and the HMQC spectrum demonstrated that only three of the protons in the molecule were attached to carbon (1 x CH_2 (δ ^1H 3.90 (d, $J = 5.2$ Hz, 2H), ^{13}C 46.4); 1 x CH (δ ^1H 8.76 (s), ^{13}C 155.9); 8 x C (δ ^{13}C 96.0, 107.9, 113.4, 120.0, 125.1, 161.8, 162.1, 163.7)). Exchangeable resonances were observed at δ 6.88 (s, 2H), 8.14 (t, $J = 5.2$ Hz, 1H), and 13.10 (s, 1H) in the ^1H NMR spectrum (DMSO-d_6) of **6**, accounting for the remaining hydrogen atoms. The COSY spectrum contained a single cross-peak confirming the scalar coupling between the aliphatic methylene protons (δ 3.90) and the exchangeable proton at δ 8.14.

A previous chemical investigation of *S. carteri* resulted in the isolation of several oroidin alkaloids, including (*Z*)-hymenialdisine (**3**) and (*Z*)-3-bromohymenialdisine (**10**).[13] The HMBC spectrum of latonduine A (**6**) contained correlations that were consistent with the presence of the 2,3-dibomo-4-alkyl-5-amido fragment found in **10**. Thus, the exchangeable resonance at δ 13.10, assigned to the pyrrole N**H**, showed HMBC correlations to carbon resonances at δ 96.0 and 120.0, assigned to C-3 and C-4, respectively, and the exchangeable resonance at δ 8.14, assigned to the N-7 amide N**H**, showed correlations to carbon resonances at δ 125.1, assigned to C-5, and 46.4, assigned to C-8. By analogy with 3-bromo hymenialdisine (**10**), the carbon resonance at δ 107.9 in the spectrum of **6** was assigned to C-2.

The remaining fragment of latonduine A (**6**) had to account for four sp^2-hybridized carbons (1 x CH, 3 x C), an isolated aromatic proton (δ 8.76), three nitrogen atoms, two equivalent exchangeable protons (δ 6.88), and five sites of unsaturation. These structural requirements could be satisfied by linking the C-4 position of the pyrrole and the C-8 methylene to adjacent carbons of a 2-aminopyrimidine fragment as shown in **6**. An HMBC correlation observed between the aromatic methine resonance at δ 8.76 (H-11) and the carbon resonance at δ 120.0, assigned to C-4, tentatively suggested the orientation of the 2-aminopyrimidine ring as shown. Additional HMBC correlations observed between the C-9 resonance at δ 163.7 and the H-11 (δ 8.76), H-8/H8′ (δ 3.90), and NH_2-15 (δ 6.88) resonances, between the C-10 resonance at δ 113.4 and the H-11 (δ 8.76) and H-8/H-8′ (δ 3.90) resonances and between the C-11 resonance at δ 155.9 and the NH_2-15 (δ 6.88) resonance, were consistent with the assigned structure **6**. The four-bond NH_2-15 to C-11 and C-9 correlations seemed to be reasonable since "W coupling" pathways existed for both long-range correlations.

The alternate structure **11** could also account for all of the spectroscopic data obtained for latonduine A. However, the biogenetic oroidin building block has a minimum four-carbon linear chain separating the amide nitrogen (N-7) and the first point of attachment (C-11) of a guanidine nitrogen. Therefore, on biogenetic grounds (Figure 1), **6** appeared to be the most likely structure for latonduine A. To verify this proposal, structure **6** was synthesized as shown in Scheme 1.

The synthesis of **6** started with amide **12** prepared according to literature procedures.[7a] Treatment of neat **12** with excess methanesulfonic acid at 35 °C for 7 days gave the cyclized product **13** in 69% yield.[7b] Hydroboration of **13** with catecholborane in THF, in the presence of a catalytic amount of $LiBH_4$, followed by oxidative workup, gave the

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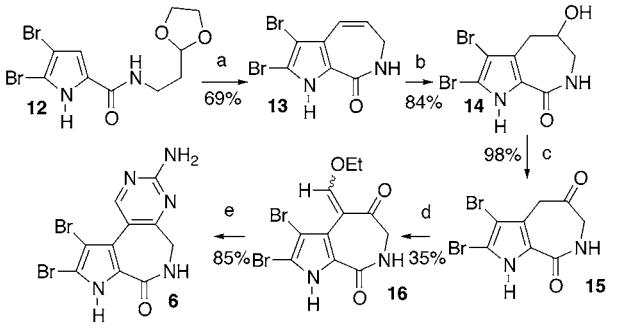
Scheme 1[a]



[a] Reaction conditions: (a) Neat, excess CH_3SO_3H, 35 °C, 7 days. (b) (i) Catecholborane, $LiBH_4$, THF, rt, 1 h; (ii) excess 1 M NaOH, 5 min; (iii) excess H_2O_2. (c) Dess−Martin periodinane (1.2 equiv), THF, rt, 2 h. (d) Excess ethylorthoformate, TFA, reflux, 18 h. (e) Guanidine, K_2CO_3, THF/H_2O 6:1, reflux, 1 h.

alcohol **14** in 84% yield. Dess−Martin oxidation of the secondary alcohol **14** gave the corresponding ketone **15** in nearly quantitative yield. Reaction of the ketone **16** with neat ethylorthoformate in the presence of TFA catalysis proceeded cleanly to give the enol ether **16** in modest yield. Treatment of **16** with excess guanidine in refluxing THF/H_2O (6:1) for 1 h gave a high yield of latonduine A (**6**), identical by NMR, MS, and TLC comparison with the natural product.

Latonduine B ethyl ester (**8**) gave a M^+ ion at *m*/*z* 444.9193 in the EIHRMS appropriate for a molecular formula of $C_{13}H_{11}N_5O_3{}^{79}Br^{81}Br$ (calcd 444.9195) that required eight sites of unsaturation. The NMR data obtained for **8** showed strong similarities to the data for latonduine A (**6**) (Supporting Information), indicating that the two compounds were closely related. The major differences in the NMR data for **8**, compared to the data for **6**, were the absence of an aromatic resonance in the region of δ 8.76, assigned to H-11 in **6**, and the presence of a complex two-proton multiplet at δ 4.19 and a three-proton triplet at 1.19, both assigned to an ethoxy fragment. An HMBC correlation from the ethoxy methylene at δ 4.19 to a carbon resonance at δ 165.0 demonstrated that the ethoxy fragment was part of an ethyl ester. The ethyl ester had to be attached at C-11 in **8** to explain the absence of the H-11 methine resonance. All of the additional two-dimensional NMR data obtained for latonduine B ethyl ester was consistent with the proposed structure **8**. Interestingly, the H-8/H-8′ protons in ester **8** gave a pair of ^{1}H NMR resonances at δ 3.77 (dd, J = 14.5, 7.1 Hz) and 4.09 (dd, J = 14.5, 3.1 Hz), indicating that an unfavorable steric interaction between the ester substituent at C-11 and the bromine at C-3 severely hinders rotation about the C-4/C-10 bond, preventing H-8 and H-8′ from interchanging rapidly on the NMR time scale. This is in contrast to the situation in latonduine A (**6**) where H-8 and H-8′ are magnetically equivalent.

The structure of latonduine B methyl ester (**9**), obtained in very small amounts from the crude extract, was routinely assigned by analysis of its spectroscopic data (Supporting Information). Isolation of the ethyl and methyl esters of

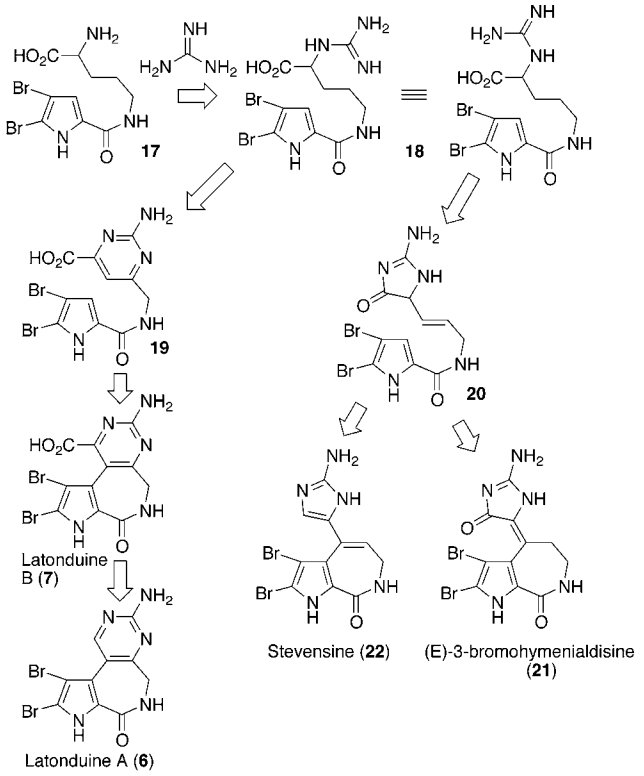


Figure 1. Proposed biogenesis of latonduines A (**6**) and B (**7**).

latonduine B from an extract of *S. carteri* that had been exposed to both ethanol and methanol during workup indicated that the free acid **7** is the natural product and that the esters are artifacts. Latonduine A (**6**) and latonduine B ethyl ester (**8**) were tested for in vitro cytotoxicity against a panel of human cancer cell lines and for enzyme inhibition against a panel of protein kinases. Both molecules were inactive in all of the assays.

Although the latonduines appear to be biogenetically related to the oroidin family of alkaloids, their skeletons cannot be derived from the $C_{11}N_5$ building block of the oroidins.[2] Instead, alkaloids **6** and **7** have an unprecedented heterocyclic skeleton that contains a six-membered aminopyrimidine substructure in the place of the five-membered aminoimidazole substructure common to the oroidin family. Figure 1 shows a proposed biogenesis for latonduine B (**7**) that suggests that the building blocks of the molecule are 4,5-dibromopyrrole-2-carboxylic acid, ornithine, and guanidine. Latonduine A (**6**) would arise from decarboxylation of lantonduine B (**7**). The biogenetic scheme also suggests that the hymenialdisines might arise from an alternate cyclization of an intermediate like **18** that involves the ornithine carboxyl functionality and a guanidine nitrogen to give the five-membered oxoaminoimidazole ring found in (*E*)-3-bromohymenialdisine (**21**).

Kerr and co-workers have shown that radiolabeled ornithine, proline, and histidine are incorporated into the hymenisaldisine analogue stevensine (**22**) in a cell culture of

the sponge *Teichaxinella morchella* but that arginine is not.[14] They proposed that ornithine was incorporated into stevensine via initial conversion to proline followed by oxidation to pyrrole-2-carboxylic acid. However, they did not degrade their radiolabeled stevensine, so the location of the ornithine label in the final molecule is only conjecture. The discovery of the latonduines, which appear to have a straightforward biogenesis from ornithine (Figure 1), suggests an alternate possibility for its incorporation in oroidin alkaloids via the aminoimidazole fragment rather than, or likely in addition to, incorporation via the pyrrolecarboxylic acid fragment. The two possible modes of incorporation of ornithine would be easily probed by doing feeding studies with oroidin (**1**), where the aminoimidazole and pyrrolecarboxylic acid fragments could be readily cleaved.

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Supporting Information Available: Experimental and NMR spectra for **6**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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