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Benzosceptrins A and B with a Unique Benzocyclobutane Skeleton and Nagelamide S and T from Pacific Sponges

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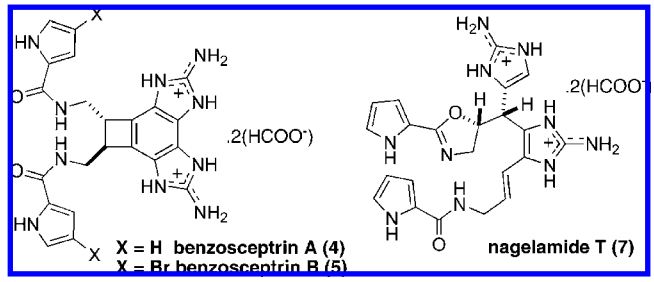
ABSTRACT



Four new dimeric pyrrole-2-aminoimidazole alkaloids have been isolated from the Pacific marine sponges *Agelas cf. mauritiana* and *Phakellia* sp. They include the unusual C2 symmetrical benzosceptrins A (4) and B (5), which each possess a unique benzocyclobutane skeleton and nagelamides S (6) and T (7). Their structures and relative configuration were elucidated from spectroscopic data. Plausible biogenetic paths for these compounds are also proposed in this paper.

A part of our previous marine metabolites research has been focused on the chemical behavior of natural pyrrole-2-aminoimidazole (P-2-AI) metabolites isolated from marine sponges belonging to the worldwide widespread Agelasidae and Axinelidae families.[1] The fabulous molecular diversity of this group of molecules is generated from the simple clathrodin (**1**),[2] hymenidin (**2**),[3] and oroidin (**3**)[4] central precursors *via* a tautomerically controlled reactivity.[5] The recent consequential developments both in isolation and synthesis have reinforced this proposal of biogenesis. Many intriguing heterocyclic dimers have been reported to co-occur with the above-mentioned three monomers, testifying to their chemical reactivity including their stereochemistry and natural chemoselectivity leading to the complexity of P-2-AI compounds.[5,6]

Our contribution focused on the structure guided isolation of additional members of these metabolites from the phylogenetically related sponges and the parallel grounding synthetic studies including the formation of the oroidin and dispacamide skeleton itself.[7]

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This communication describes the isolation of new dimers **4**−**7** from the sponges *Agelas cf. mauritiana* and *Phakellia* sp. and proposes plausible biogenetic chemical pathways for their origin.

The sponge *Agelas cf. mauritiana*[7c,8] (Demospongiae, order Agelasida, family Agelasidae) collected on northern Guadalcanal reefs off the Solomon Islands was extracted with dichloromethane and then with methanol. The methanolic crude extract was partitioned between water and *n*-BuOH. *n*-BuOH-soluble material was purified by silica gel chromatography followed by repeated C_{18} HPLC to yield benzosceptrin A (**4**), nagelamide S (**6**), and nagelamide T (**7**) as formate salts (Figure 1). Benzosceptrin B (**5**) was obtained from the sponge,

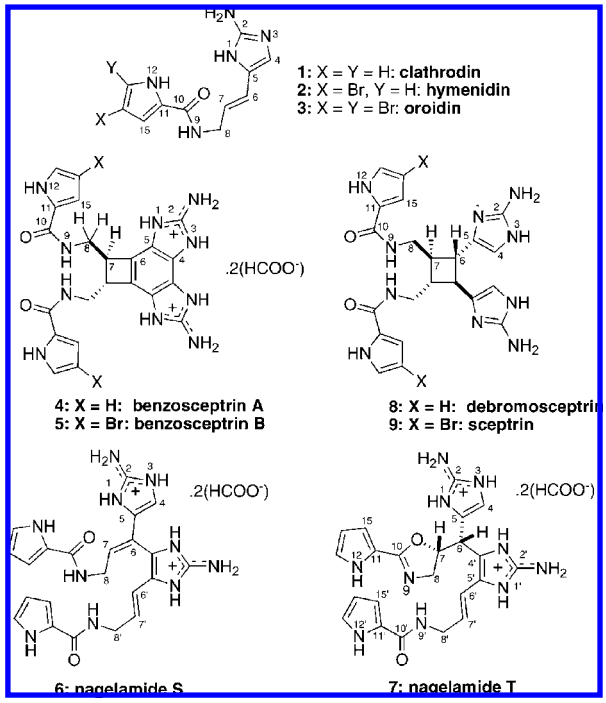

Figure 1. New dimeric molecules **4**−**7** isolated from the pacific sponges and related known P-2-AIs **1**−**3** and **8**.

Phakellia sp. (order Halichondrida, family Axinellidae) collected off New Caledonia, by the same procedure.

The LCMS and ESIMS spectra of **4**, **6**, and **7** indicated that the three compounds were not brominated as is usually observed for the pyrrole-2-aminoimidazole metabolites. ^{1}H NMR and UV spectra showed the presence of the characteristic protons of the non-substituted pyrrole 2-carboxamide derivatives.

The molecular formula of benzosceptrin A (**4**) (0.5 mg, 0.0001%)[9] was established to be $C_{22}H_{22}N_{10}O_2$ by HRESIMS data [*m*/*z* 459.1996 $(M + H)^+$]. The 600 MHz ^{1}H NMR spectrum of **4** (Table 1) appears to be rather simple. In addition to three pyrrole protons, only three signals integrating for two protons each (δ_H = 3.54, 3.69, and 3.95 in CD_3OD) could be observed. As for the sceptrin and debromosceptrin (**8**),[10] this suggested the presence of a symmetric structure. Comparison of the formula of the debromosceptrin with compound **4** showed that the latter had two unsaturations more than debromosceptrin and that both H-4 and H-6

Table 1. ^{1}H and ^{13}C NMR Data (600 MHz) for Benzosceptrins A (**4**) and B (**5**) in CD_3OD

	4		5	
position[b]	δ_H	δ_c	δ_H	δ_c
2		154.2		155.3
4		124.1/123.6		124.0
5		123.6/124.1		126.1
6		120.6		121.2
7	3.69 (dd, 8.7, 4.3)	49.9[a]	3.67 (dd, 8.6, 4.0)	48.2[a]
8	3.95 (dd, 13.4, 4.3)	43.2	3.93 (dd, 11.0, 4.0)	43.9
	3.54 (dd, 13.4, 8.7)		3.53 (dd, 11.0)	
10		164.1		162.8
11		126.6		127.0
13	6.86 (dd, 2.6, 1.4)	122.9	6.86 (dd, 2.4)	122.8
14	6.08 (dd, 3.8, 2.6)	110.3		97.3
15	6.61 (dd, 3.8, 1.4)	112.1	6.61 (dd, 3.8)	113.4

[a] Overlapped with solvent signal and deducted from HSQC correlation. [b] Because **4** and **5** are C2 symmetric, 1 stands for 1 and 1′; 2 stands for 2 and 2′, etc.

protons were missing from the ^{1}H NMR spectrum. This data suggested the presence of a benzene ring (three sp^2 quaternary carbons at δ_C 120.6, 123.6, and 124.1) linking the 2-aminoimidazole rings to the C-4 position. The remaining sp^2 quaternary carbon (δ_C 126.6) was assigned to C-11 in proportion to its HMBC correlation with H-15 (Figure 2).

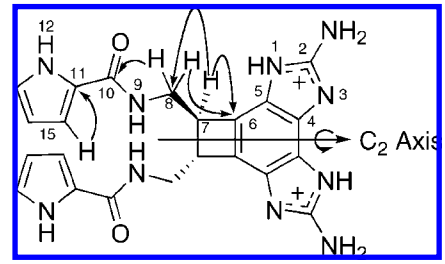

Figure 2. Selected HMBC correlations for **4**.

The HMBC correlation between the H-7 signal and the benzene quaternary carbon C-6 could arise only if two 2-aminoimidazoles and the cyclobutane were bonded together by a benzene ring. Furthermore, the H_2-8 signals showed correlations with the quaternary carbons C-6 and C-10 of the carbonyl group (Figure 2). At this stage, the weak optical rotation of **4** ($[\alpha]^{25}_D$ −1.8 (*c* 0.05, MeOH)) left room for a C2 symmetry axis or the *meso* diastereoisomer.

Further, we could also confirm the structure of **4** by comparison with the second C2 symmetrical benzosceptrin B (**5**)[11] that we have isolated from another New Calodonian sponge, *Phakellia* sp. The ESI-MS of **5** displayed pseudomoleular ion peaks at *m*/*z* 614, 616, and 618 in the ratio 1:2:1 suggesting the presence of two bromine atoms in the molecule. The molecular formula was revealed as $C_{22}H_{20}Br_2N_{10}O_2$ from HRESI-MS (*m*/*z* 615.0201 $(M + H)^+$, Δ1.62 ppm). The 600 MHz ^{1}H and ^{13}C NMR data has been given in Table 1. The 600 MHz ^{1}H NMR spectra of **4** and **5** were superimposable, except for the absence of pyrrole proton peak at δ_H 6.08 for

H-14 in **5**, which is replaced by bromine atom. The ^{13}C NMR spectrum gave the corresponding carbon value at δ_C 97.3 for C-14 (δ_C 110.3 in **4**). This confirmed the presence of monobromopyrrole moiety in the molecule. The carbons shifts at δ_C 124.0 (C-4), 126.1 (C-5), and 121.2 (C-6) further supported the occurrence of the aromatic ring in the molecule. Therefore, the structure was confirmed to be dibrominated metabolite, benzosceptrin B. The specific rotation, $[\alpha]^{25}_D$ + 23.4, for **5** and the similarity of its NMR spectra to those of benzosceptrins A (**4**) suggested that both of the molecules had a *trans* configuration at C-7 and C-7′ and thus had a C2 symmetry axis as for sceptrins **8** and **9**.

The molecular formula of **6** (3 mg, 0.0006%) was established to be $C_{22}H_{24}N_{10}O_2$ by HRESI-MS data [*m/z* 459.2015 $(M - H)^-$]. Inspection of the 600 MHz 1D and 2D NMR spectra in CD_3OD indicated two different olefins: an *E*-disubstituted alkene with its protons at $\delta_H = 6.04$ (dt, $J = 15.8$, 5.3 Hz and $\delta_H = 6.13$ (dt, $J = 15.8$ Hz) and a trisubstituted at $\delta_H = 6.12$ (t, $J = 7.0$ Hz). The presence of only one characteristic imidazolic proton at $\delta_H = 6.45$ (s) was consistent with two oroidin type subunits with a C4′−C6 linkage, a structural feature found in nagelamides A−D,[12] J,[13] K[14] and R[15] isolated from *Agelas* sponges by Kobayashi and co-workers. Further, comparison of the spectral data of the nagelamide C[12] with our new compound led to the conclusion that it was non-brominated nagelamide C. To avoid any confusion due to the more or less brominated derivatives in the literature, **6** is called nagelamide S. The molecular formula of nagelamide T (**7**) was revealed to be $C_{22}H_{24}N_{10}O_2$ by HRESI-MS data [*m/z* 459.1983 $(M - H)^-$, Δ −4.9 mmu] indicating that **7** is a mass isomer of **6**. 600 MHz ^1H NMR data for **7** in CD_3OD (see Supporting Information) showed three olefinic protons, two belonging to an *E*-disubstituted double bound ($\delta_H = 5.97$, dt, $J = 16.1$, 5.8 Hz and $\delta_H = 6.39$, d, $J = 16.1$ Hz) and one imidazole proton ($\delta_H = 6.78$, s). This was consistent with an oroidin-type subunit with a C4′−C6 linkage to a second modified oroidin moiety (Figure 1). The nature of the C6−C8 sequence was determined as follows: the lack of a C6−C7 double bound and the presence of two *gem*-coupling protons ($\delta_H = 4.10$, 3.77, dd) suggested the presence of an additional heterocycle. The $^1H-^1H$ COSY correlations led to a cyclic structure that included C7 and C8 with C6 in the bisallylic position ($\delta_H = 4.26$, d). The chemical shift for H-7 ($\delta_H = 5.25$, m) could be attributed to a methine linked to an oxygen. The comparison of these value with those of nagelamide R indicated the presence of an oxazoline ring. The planar structure of **7** was confirmed by additional 2D NMR experiments in CD_3OD, and the *R* configuration for C-7 was assigned by NOESY analysis (Figure 3). It should be noted

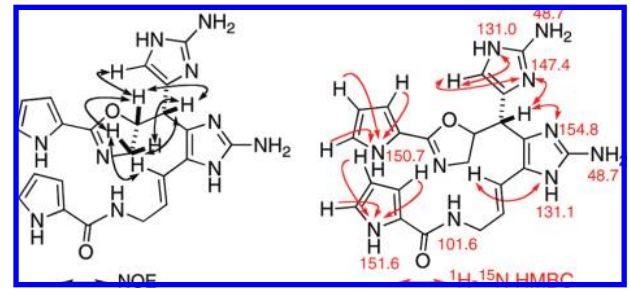

Figure 3. Selected NOESY and $^1H-^{15}N$ HMBC correlations for nagelamide T (**7**) in CD_3OD; δ ppm for ^{15}N are indicated in red.

that 600 MHz $^1H-^{15}N$ HMBC NMR experiments were performed in order to indicate the positions of the protons for both 2-aminoimidazole parts.[16]

These data together with the a comparison to nagelamide R were consistent with **7** being the non-brominated derivative of nagelamide R that we have named nagelamide T (**7**).

A plausible biogenetic path for benzosceptrin A and B from the naturally occurring debromosceptrin (**8**) and sceptrin (**9**) is proposed as shown in Scheme 1. The tautomerism of the 2-aminoimidazole section[5,7b] could afford a cyclization step followed by aromatization to yield benzosceptrins A and B (path a). However, AM1 calculations[17] for the transformation of debromosceptrin into benzosceptrin A through their formation enthalpies revealed that +46.46 kcal is required. This result may be intuitively obvious regarding the *trans* cyclobutene substitution, but the enzymatic catalyzed biosynthetic reactions and the apparent strong role of the tautomerism in P-2-AI compounds would make the transformation possible. Nevertheless, the preformed cyclobutane, when starting from sceptrin-like compounds, brings the 2-aminoimidazole rings to unfavorable positions where the high energy cost could prohibit the benzene ring formation through C4−C4′ bound formation. Thus, a second

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(9) Benzosceptrin A (**4**): white amorphous solid; $[\alpha]^{25}_D$ −1.8 (*c* 0.05, MeOH); UV λ_{max}= 203, 222 and 265 nm; IR (neat) ν_{max} 3340, 1632, 1566, 1408, 1337, 1201, 1118 and 1043 cm^{-1}; (+) HRMS-ESI *m/z* 459. 1996 $[(M + H)^+$, Δ −0.9 mmu, calcd for $C_{22}H_{23}N_{10}O_2$ *m/z* 459.2005].

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(11) Benzosceptrin (**5**): colorless amorphous solid; $[\alpha]^{25}_D$ + 23.4 (c 0.1; MeOH); UV λ_{max}= 201, 216, 268 nm; IR (neat) ν_{max} 3345, 2359, 1632, 1656, 1023, 921 cm^{-1}. (+) HRESI-MS *m/z* 615.0201, $[(M + H)^+$, calcd for $C_{22}H_{20}Br_2N_{10}O_2$ *m/z* 615.0211].

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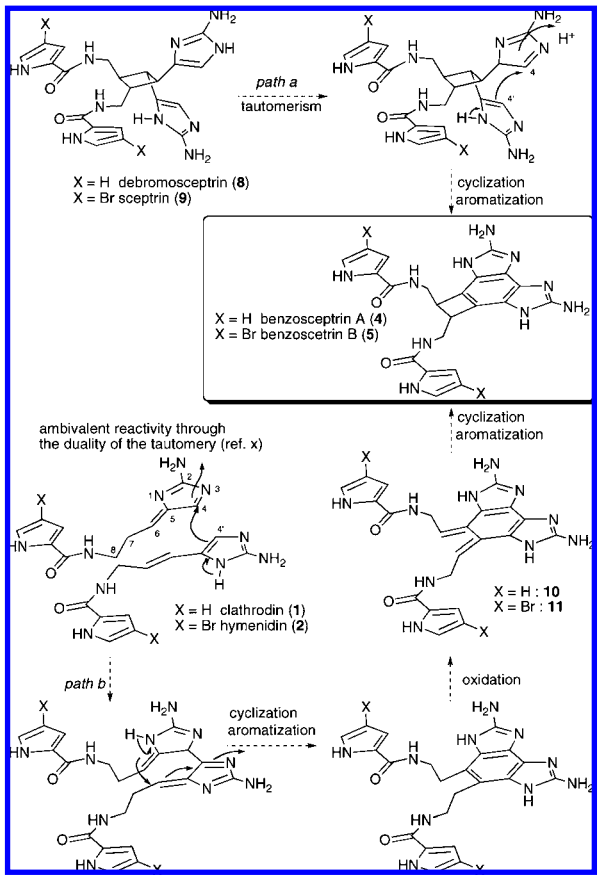
Scheme 1. Plausible Biogenetic Paths for Benzosceptrins A (**4**) and B (**5**) from Debromosceptrin (**8**) and Sceptrin (**9**)

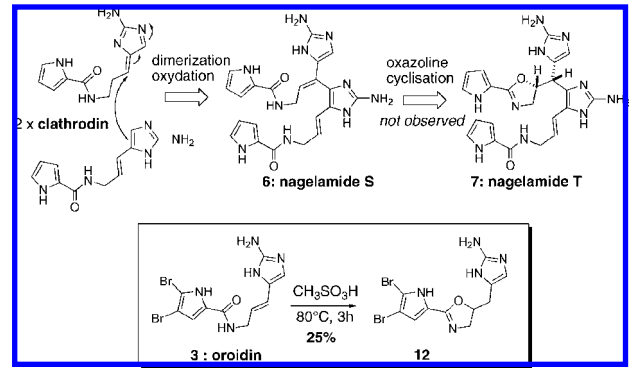


pathway implying steps with lower energy levels and considering benzene ring formation at first was suggested. The ambivalent reactivity through the duality of the tautomerism leaves enough room for other possibilities starting from the monomers clathrodin and hymenidin.

The dimerization/cyclization/oxidation products rising from a P-2-AI monomer like clathrodin (**1**) reacting with itself could afford a very large subclass of dimers. Regarding the benzosceptrin A, tautomerization dimerization/cyclization from clathrodin following path b can lead to the energetically reasonable intermediate **10** were the benzene cycle is formed prior to the cylobutene as in debromosceptrin. The formation enthalpy of benzosceptrin A (**4**) from the putative intermediate **10** requires 11.05, kcal which is about 4 times lower than when starting from debromosceptrin (**8**). The cyclization/oxidation of **10** into **4** as for the majority of the formation of the polycyclic P-2-AI dimers must be accompanied by a formal oxidation as well. The oxidation steps seem to play an important role not only for the cascade progression but also for stabilization of the final compounds, otherwise the dynamic tautomerism could lead to a quick degradation of the intermediates.

Following from the tautomerism of monomers presented by our group as a universal pathway explaining the molecular diversity of the P-2-AIs, a biogenetic path for nagelamides S and T starting from clathrodin (**1**) can be proposed. After dimerization and oxidation, nagelamide S could be formed (Scheme 2). Nagelamide S could be the precursor of nagelamide

Scheme 2. Plausible Biogenetic Path for Nagelamides S (**6**) and T (**7**) from Clathrodin and Formation of Oxazoline (**12**) from Oroidin



T through the formation of an oxazoline. The transformation of oroidin (**3**) is possible without additional oxidation in CH_3SO_3H. The oxazoline **12** was obtained in 25% yield (Scheme 2). However, attempts to convert nagelamide S (**6**) into nagelamide T (**7**) under the same conditions failed.

In conclusion, isolation of benzosceptrins A (**4**) and B (**5**) appears particularly remarkable since it represents the first report of benzocyclobutane containing compounds from natural sources. Benzosceptrin A (**4**) and B (**5**) show an unreported C2 symmetrical benzocyclobutane, and nagelamides S (**6**) and T (**7**) are two non-brominated analogs of nagelamides C and R, respectively. Plausible biogenetic paths for these dimers have been proposed starting from debromosceptrin and clathrodin. These new compounds again strengthen our biosynthetic proposal for marine pyrrole-2-aminoimidazole (P-2-AI) metabolites.

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Supporting Information Available: Extraction procedure, isolation and characterization data (including ^{1}H and ^{13}C NMR spectra) for compounds **4**−**7** and **12**. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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