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

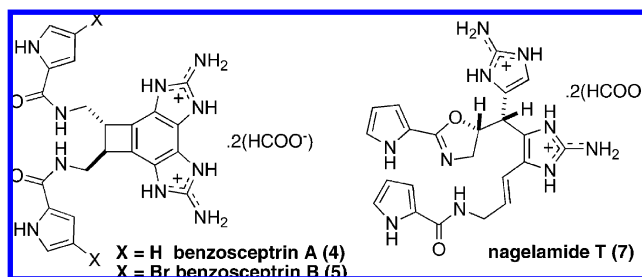
Jérôme Appenzeller,[†] Supriya Tilvi,[†] Marie-Thérèse Martin,[†]
Jean-François Gallard,[†] Hoda El-bitar,[†] Elise Tran Huu Dau,[†] Cécile Debitus,[‡]
Dominique Laurent,[§] Céline Moriou,[†] and Ali Al-Mourabit^{*,†}

Centre de Recherche de Gif-sur-Yvette, Institut de Chimie des Substances Naturelles,
UPR 2301, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France, IRD-UMR 152,
Centre polynésien de recherche sur la biodiversité insulaire, Papeete, French Polynesia,
and UMR 152 IRD-Université Paul Sabatier Toulouse III, 31062 Toulouse cedex 9, France

ali.almourabit@icsn.cnrs-gif.fr

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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 tautomatically 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

[†] Institut de Chimie des Substances Naturelles.

[‡] Centre polynésien de recherche sur la biodiversité insulaire.

[§] UMR 152 IRD-Université Paul Sabatier Toulouse II.

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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 C₁₈ 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,

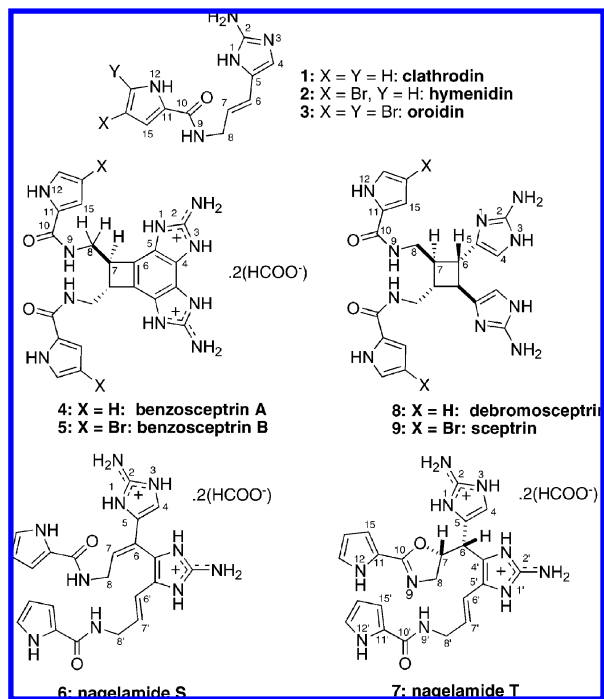


Figure 1. New dimeric molecules **4**–**7** isolated from the pacific sponges and related known P-2-AIs **1**–**3** and **8**.

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. ¹H 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 C₂₂H₂₂N₁₀O₂ by HRESIMS data [*m/z* 459.1996 (*M* + *H*)⁺]. The 600 MHz ¹H 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 CD₃OD) 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. ¹H and ¹³C NMR Data (600 MHz) for Benzosceptrins A (**4**) and B (**5**) in CD₃OD

position ^b	4		5	
	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 ¹H NMR spectrum. This data suggested the presence of a benzene ring (three sp² quaternary carbons at δ_{C} 120.6, 123.6, and 124.1) linking the 2-aminoimidazole rings to the C-4 position. The remaining sp² quaternary carbon (δ_{C} 126.6) was assigned to C-11 in proportion to its HMBC correlation with H-15 (Figure 2).

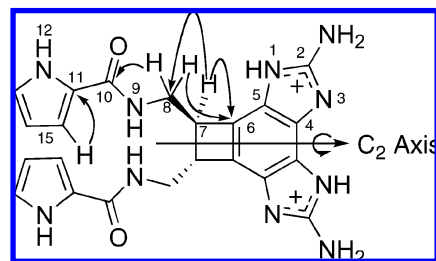


Figure 2. Selected HMBC correlations for **4**.

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 H₂-8 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** ($[\alpha]_{\text{D}}^{25} -1.8$ (*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 pseudo-molecular 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 C₂₂H₂₀Br₂N₁₀O₂ from HRESI-MS (*m/z* 615.0201 (*M* + *H*)⁺, Δ 1.62 ppm). The 600 MHz ¹H and ¹³C NMR data has been given in Table 1. The 600 MHz ¹H 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, $[\alpha]_{\text{D}}^{25} + 23.4$, 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 $\text{C}_{22}\text{H}_{24}\text{N}_{10}\text{O}_2$ by HRESI-MS data [m/z 459.2015 ($\text{M} - \text{H}$) $^-$]. 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 $\delta_{\text{H}} = 6.04$ (dt, $J = 15.8, 5.3$ Hz and $\delta_{\text{H}} = 6.13$ (dt, $J = 15.8$ Hz) and a trisubstituted at $\delta_{\text{H}} = 6.12$ (t, $J = 7.0$ Hz). The presence of only one characteristic imidazolic proton at $\delta_{\text{H}} = 6.45$ (s) was consistent with two oroidin type subunits with a C4'–C6 linkage, a structural feature found in nagelamides A–D,¹² J,¹³ K¹⁴ and R¹⁵ isolated from *Agelas* sponges by Kobayashi and co-workers. Further, comparison of the spectral data of the nagelamide C¹² 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 $\text{C}_{22}\text{H}_{24}\text{N}_{10}\text{O}_2$ by HRESI-MS data [m/z 459.1983 ($\text{M} - \text{H}$) $^-$, $\Delta -4.9$ 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 bond ($\delta_{\text{H}} = 5.97$, dt, $J = 16.1, 5.8$ Hz and $\delta_{\text{H}} = 6.39$, d, $J = 16.1$ Hz) and one imidazole proton ($\delta_{\text{H}} = 6.78$, 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 bond and the presence of two *gem*-coupling protons ($\delta_{\text{H}} = 4.10, 3.77$, 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 ($\delta_{\text{H}} = 4.26$, d). The chemical shift for H-7 ($\delta_{\text{H}} = 5.25$, 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

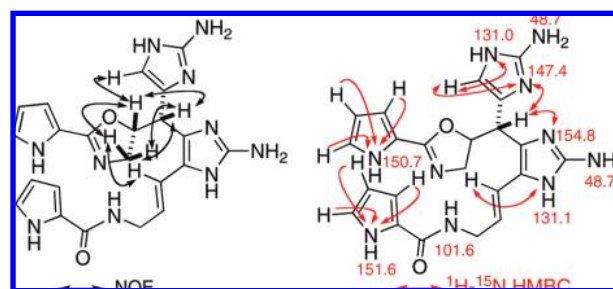


Figure 3. Selected NOESY and ^1H – ^{15}N HMBC correlations for nagelamide T (**7**) in CD_3OD ; δ ppm for ^{15}N are indicated in red.

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 calculations¹⁷ 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' bond formation. Thus, a second

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(9) Benzosceptrin A (**4**): white amorphous solid; $[\alpha]_{\text{D}}^{25} -1.8$ (c 0.05, MeOH); UV $\lambda_{\text{max}} = 203, 222$ and 265 nm; IR (neat) $\nu_{\text{max}} 3340, 1632, 1566, 1408, 1337, 1201, 1118$ and 1043 cm^{-1} ; (+) HRMS-ESI m/z 459. 1996 [(M + H) $^+$, $\Delta -0.9$ mmu, calcd for $\text{C}_{22}\text{H}_{23}\text{N}_{10}\text{O}_2$ m/z 459.2005].

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(11) Benzosceptrin (**5**): colorless amorphous solid; $[\alpha]_{\text{D}}^{25} + 23.4$ (c 0.1; MeOH); UV $\lambda_{\text{max}} = 201, 216, 268$ nm; IR (neat) $\nu_{\text{max}} 3345, 2359, 1632, 1656, 1023, 921$ cm^{-1} . (+) HRESI-MS m/z 615.0201, [(M + H) $^+$, calcd for $\text{C}_{22}\text{H}_{20}\text{Br}_2\text{N}_{10}\text{O}_2$ m/z 615.0211].

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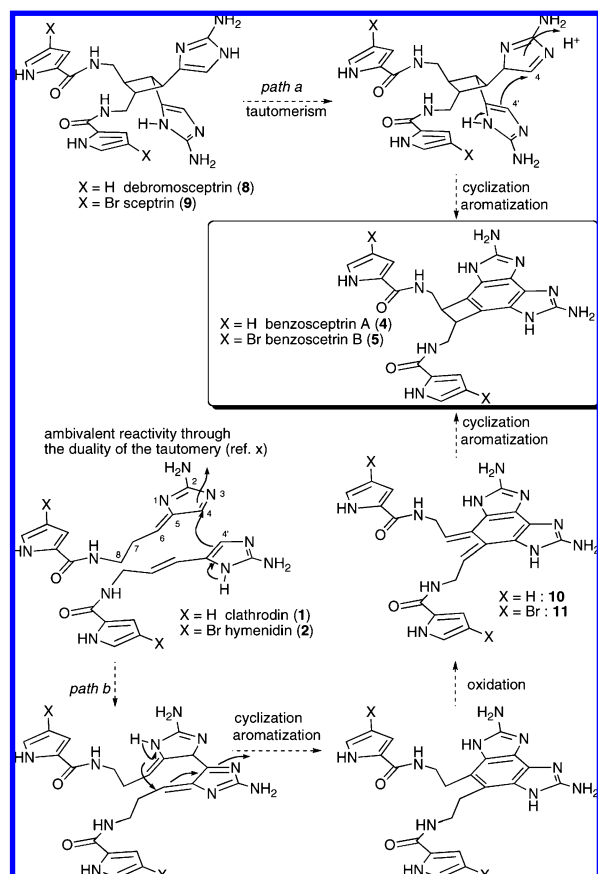
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Scheme 1. Plausible Biogenetic Paths for Benzosceptrins A (4) and B (5) from Debromosceptrin (8) and Sceptrin (9)



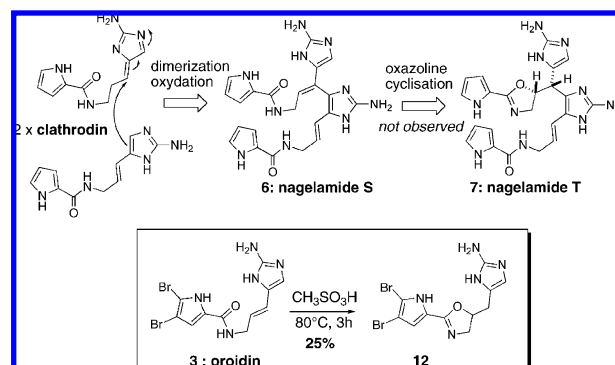
pathway implying steps with lower energy levels and considering benzene ring formation at first was suggested. The ambivalent reactivity through the duality of the tautomerism leaves enough room for other possibilities starting from the monomers clathrocin and hymenidin.

The dimerization/cyclization/oxidation products rising from a P-2-AI monomer like clathrocin (1) reacting with itself could afford a very large subclass of dimers. Regarding the benzosceptrin A, tautomerization dimerization/cyclization from clathrocin following path b can lead to the energetically reasonable intermediate **10** where the benzene cycle is formed prior to the cyclobutene 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 clathrocin (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 Clathrocin 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 $\text{CH}_3\text{SO}_3\text{H}$. 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 clathrocin. 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 ^1H 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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