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Debromodispacamides B and D: Isolation from the Marine Sponge *Agelas mauritiana* and Stereoselective Synthesis Using a Biomimetic Proline Route

Carine Vergne,[†] Jérôme Appenzeller,[†] Céline Ratinaud,[†] Marie-Thérèse Martin,[†] Cécile Debitus,[‡] Anne Zaparucha,[†] and Ali Al-Mourabit*,[†]

Institut de Chimie des Substances Naturelles du CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France, and UMR 152 IRD-Université Paul Sabatier Toulouse III, Faculté des Sciences Pharmaceutiques, 31062 Toulouse cedex 9, France

ali.almourabit@icsn.cnrs-gif.fr

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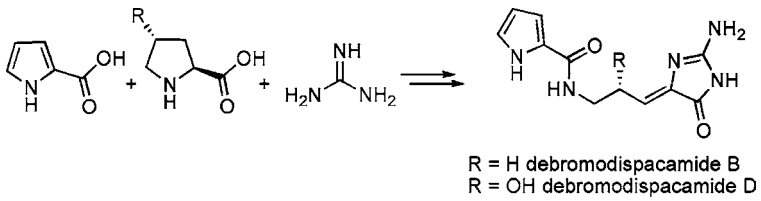
ABSTRACT



New debromopyrrole-2-aminoimidazolones, debromodispacamide B (1) and debromodispacamide D (2) were isolated from the sponge *Agelas mauritiana,* collected in the Solomon Islands. A biomimetic one-step reaction from pseudopeptides 5 and 13 in presence of air oxygen and guanidine gave the chiral form of the natural product stereoselectively.

Marine sponges are a rich source of bromopyrrole alkaloids.[1] In particular the sponge families Agelasidae and Axinellidae widely contain brominated pyrrole-2-aminoimidazole alkaloids.[2] As part of our ongoing biogenetic investigations through the exhaustive isolation and synthesis of the pyrrole-2-aminoimidazole (P-2-AI) metabolite family, we investigated the polar extract of the sponge *Agelas mauritiana*. collected off the Solomon Islands. Here we described the isolation and the biomimetic synthesis of two new non-brominated P-2-AI belonging to the dispacamide family.

Specimens of *Agelas mauritiana* (Demospongiae, order Agelasida, family Agelasidae) were collected by scuba divers on Guadalcanal reefs off the Solomon Islands (18 to 30 m depth, June 2004). Freshly collected sponges were frozen and lyophilized. The sponge was sucessively extracted with dichloromethane and methanol. Methanolic crude extract was partitioned between *n*-butanol and water. The *n*-butanol-soluble material was subjected to silica gel column chromatography using dichloromethane/methanol gradient as eluent. The fraction containing P-2-AI alkaloids obtained with 15% methanol in dichloromethane was further purified by preparative HPLC to yield 3 mgeach of debromodispacamide B (**1**) and debromodispacamide D (**2**) (Figure 1).

Debromodispacamide B (**1**) presents a HRMS $(M + H)^+$ ion at *m*/*z* 248.1122, indicating the formula of $C_{11}H_{12}N_5O_2$. The ^1H NMR spectrum showed strong similarities to the data

[†] Institut de Chimie des Substances Naturelles du CNRS.
[‡] UMR 152 IRD-Université Paul Sabatier Toulouse III, Faculté des Sciences Pharmaceutiques.

(1) (a) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48. (b) Al-Mourabit, A.; Potier, P. *Eur. J. Org. Chem.* **2001**, 237−243.
(2) (a) Braeckman, J.-C.; Daloze, D.; Stoller, C.; Van Soest, R. W. M. *Biochem. Syst. Ecol.* **1992**, *20*, 417−431. (b) Erpenbeck, D.; van Soest, R. W. *Mar. Biotechnol.* **2007**, *9*, 2−19.

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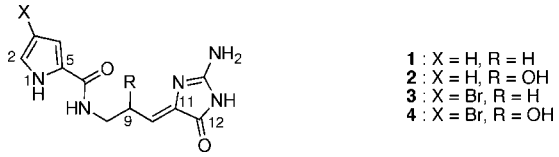


Figure 1. Debromodispacamides and dispacamides B and D.

for the known compound dispacamide B (**3**) isolated from an *Agelas* sp.[3] The only differences between **1** and **3** were the presence of the pyrrolic H-3 at 6.17 ppm besides H-2 and H-4 appearing as doublets of doublets at 6.86 and 6.72 ppm, respectively, indicating the presence of a nonsubstituted pyrrole unit. The HMBC correlation of the olefinic proton H-10 at 5.71 ppm with C-12 at 168.4 ppm and the coupling constant ($^3J_{C-H}$ = 4.3 Hz) confirmed the *cis* relationship between the olefinic proton and the carbonyle function, meaning a (***Z***) configuration of the double bond. The configuration of the exocyclic double bound was correlated with the $^3J_{C-H}$ coupling constants by Branko Stanovnik and co-workers.[4] The systematically measured coupling constants of various imidazolones were between 2 and 6 Hz for nuclei with *cis*-configuration ((*Z*)-isomers), instead between 8 and 12 Hz for nuclei with *trans*-configuration ((*E*)-isomers) (Figure 2).

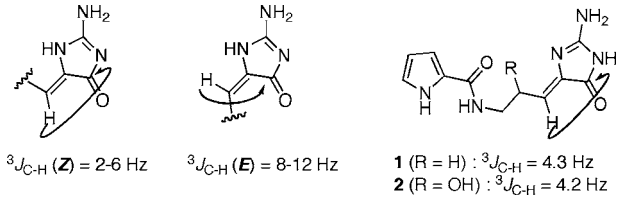
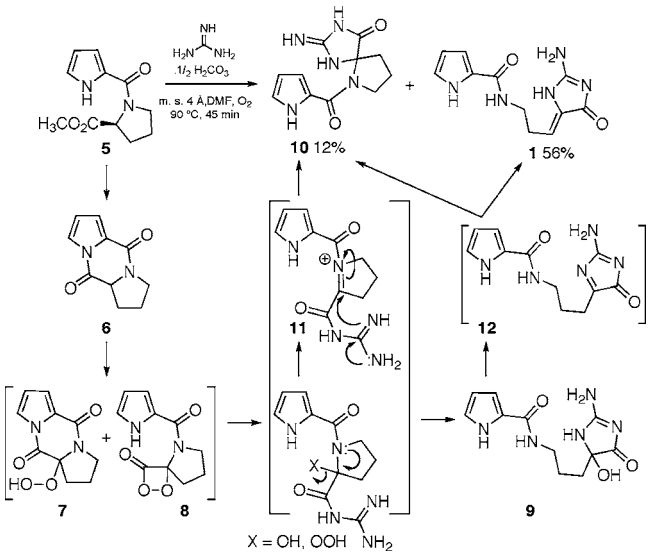


Figure 2. Comparison of $^3J_{C-H}$ coupling constants of (***E***) and (***Z***)-isomers and the (***Z***) configuration of **1** and **2**.

The molecular formula of debromodispacamide D (**2**) was confirmed as $C_{11}H_{12}N_5O_3$ by HRMS. The comparison of NMR spectral data with the data of the known brominated dispacamide D (**4**)[5] and its two-dimensional (2D) NMR analysis gave the non-brominated and hydroxylated molecule **2**. The configuration of the double bond was similarly assigned to be (*Z*) ($^3J_{C-H}$ = 4.2 Hz). The only uncertainty remaining to be solved was the configuration of C-9 of the side chain. The specific rotation $[\alpha]^{25}_D$ 0° (*c* = 0.28, MeOH) indicated that **2** was a racemate that was confirmed by the stereoselective synthesis described in the following section of this letter.

The synthesis of **1** and **2** was designed according to our previous proposed biogenetic hypothesis based on the new oxidative transformation of the pyrrole-proline pseudopeptide **5** and guanidine into hydroxyimidazolidinone **9** presenting the pyrrole-2-aminoimidazole skeleton (Scheme 1).[6]

Scheme 1. Debromodispacamide B Synthesis



The mechanistic studies regarding the general oxidative transformations of pseudopeptide derivatives such as **5** will be published elsewhere.

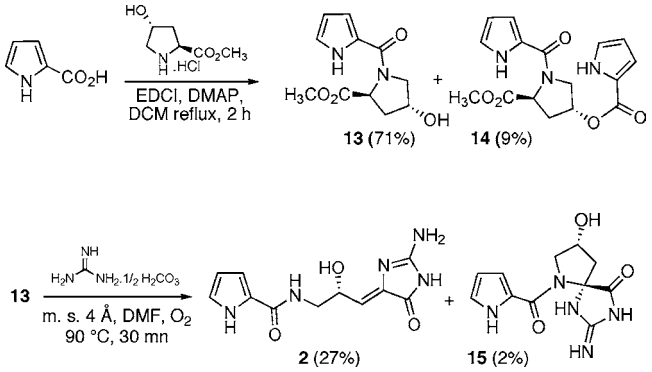
During our survey, we found that the one-pot reaction of the pseudopeptide pyrrole-proline methyl ester **5** with air oxygen in the presence of guanidine carbonate and 4 Å molecular sieves, in DMF at 90 °C for 45 min, afforded stereoselectively the desired debromodispacamide B (**1**) in 56% yield, together with the spiropyrrolidine aminoimidazolone[7] **10** in 12% yield (Scheme 1). The mechanism of the reaction could be explained through the formation of the intermediates **11** or **12** followed by the nucleophilic attack of the guanidinic or amidic nitrogens, respectively (Scheme 1).

Following the same strategy, the stereospecific synthesis of the (+)-debromodispacamide D (**2**) started with the pseudodipeptide pyrrole-hydroxyproline methyl ester **13**. Reaction of the commercially available *trans*-L-4-hydroxyproline methyl ester with the 2-pyrrolecarboxylic acid in the presence of a coupling reagent (EDCI) gave the pseudodipeptide **13** together with the *O*-acylated product **14**, in 71% and 9% yields, respectively. The reaction of the guanidine carbonate with **13** in DMF in the presence of 4 Å molecular sieves at 90 °C for 30 min led to the formation of

(3) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1996**, *37*, 3587−3590.
(4) Jakse, R.; Recnik, S.; Svete, J.; Golobic, A.; Golic, L.; Stanovnik, B. *Tetrahedron* **2001**, *57*, 8395−8403.
(5) Cafieri, F.; Carnussio, R.; Fattorusso, E.; Taglialatela-Scafati, O.; Vallefuoco, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2283−2288.
(6) (a) Travert, N.; Al-Mourabit, A. *J. Am. Chem. Soc.* **2004**, *126*, 10252−10253. (b) Vergne, C.; Boury-Esnault, N.; Perez, T.; Martin, M.-T.; Adeline, M.-T.; Tran Huu Dau, E.; Al-Mourabit, A. *Org. Lett.* **2006**, *8*, 2421−2424.
(7) For the synthesis of spiropyrrolidine imidazolones see : Fresneda, P. M.; Castaneda, M.; Sanz, M. A.; Bautista, D.; Molina, P. *Tetrahedron* **2007**, *63*, 1849−1856.

debromodispacamide D (**2**) as the (*R*) enantiomer in 27% yield, together with the spiropyrrolidine aminoimidazolone **15** in 2% isolated yield (Scheme 2).

Scheme 2. Debromodispacamide D Synthesis



The stereochemistry of the minor compound **15** was assigned according to NOESY experiments (Figure 3). Its

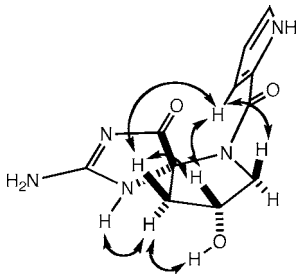


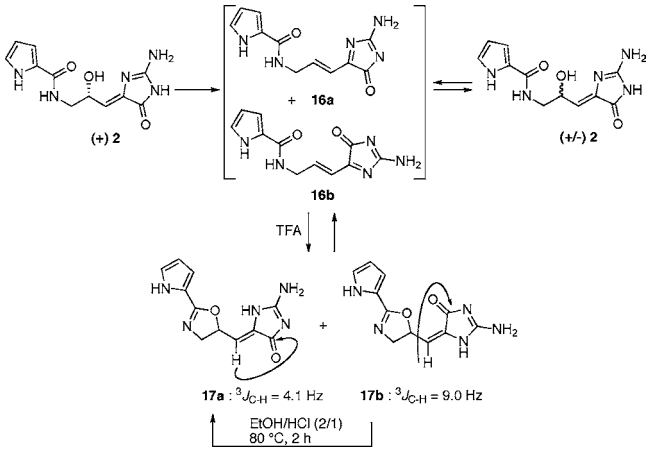
Figure 3. Stereochemistry of the spirocompound **15**. NOESY correlations.

formation could be explained by a similar mechanism as for **10** (Scheme 1).

The optical rotation obtained for the synthetic material of (+)-debromodispacamide D (**2**) $[\alpha]^{25}_D$ +41.7° (*c* = 0.34, MeOH) indicated that the natural material $[\alpha]^{25}_D$ 0° (*c* = 0.28, MeOH) that we have isolated was a racemate. The optical rotations of the other natural members of natural dispacamide family indicated that the absolute configuration at C9 is not stable. The examples of the brominated derivatives dispacamides C and D described by Fattorusso ($[\alpha]_D$ 0°)[5] and tauroacidins A ($[\alpha]_D$ −4.3°) and B ($[\alpha]_D$ +5°)[8] are in favor of a racemization during the purification processes. Kobayashi has even described the same dipacamide D (=mukanadin A, $[\alpha]_D$ +5°)[9] for a 7:3 mixture of enantiomers.

We wanted to probe the stability of the chirality of (+)-debromodispacamide D (**2**) in an acidic condition. Treatment with trifluoroacetic acid of **2** (50 mg) at 60 °C gave a complex mixture of compounds from which the 1/1 mixture of oxazolines (***Z***)-**17a** ($^3J_{C-H}$ = 4.1 Hz) and (***E***)-**17b** ($^3J_{C-H}$ = 9.0 Hz) were isolated in 21% yield (Scheme 3). The structures were determined by 2D-NMR

Scheme 3. Racemization of the Natural Compound



including coupling constants mesurements as for the natural products **1** and **2** described above.

If the pH-dependent isomerization takes place through **16a** and **16b**, then the hydrolysis of the oxazolines **17a** and **17b** should lead to the (±)**2**. To unambiguously confirm the common pathway, **17a** and **17b** were subjected to various attempts of hydrolysis/isomerization. By heating the 1/1 mixture (***Z***)-**17a** + (***E***)-**17b** in EtOH/1 N HCl: (2/1) at 60 °C for 2.5 h, (***E***)-**17b** was transformed into (***Z***)-**17a** quantitatively. Regarding the latter result, it is tempting to consider that the transient isomers **16a** and **16b** play an important role in both formation of oxazolines and racemization. This may explain why the natural product is racemic. The simple experiment consisting in the stirring of the synthetic material (+)**2** in 6 N HCl for 2 h at room temperature led to a complex reaction mixture. To prevent the degradation, the same experiment was repeated for 15 min at room temperature. After the purification, the recovered starting material **2** (60% yield) showed a similar $[\alpha]^{25}_D$ +40° (*c* = 1.29, MeOH) as for the starting material. Finaly, the experiments that we have conducted on the synthetic material do not support any clean racemization in acidic or basic conditions. The degradation of the starting material is the major process that we have observed.

In summary, we have isolated two new non-brominated pyrrole-2-aminoimidazolone metabolites **1** and **2** from the marine sponge *Agelas mauritiana* collected in the Solomon Islands. The straightforward stereoselective synthesis reported here underlines the likelihood of our biogenetic hypothesis based on the oxidative rearrangement of the proline and hydroxyproline units. We believe that the formation of pyrrole-2-aminoimidazolones metabolites from pyrrole con-

(8) Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1999**, *49*, 16679−16682.

(9) Dispacamide D described in ref 5 has been named mukanadin A (2 years later)! Uemoto, H.; Tsuda, M.; and Kobayashi, J. *J. Nat. Prod.* **1999**, *62*, 1581−1583.

taining diketopiperazines by spontaneous air oxidation and skeletal rearrangement is of high significance for the biosynthesis of this family of marine metabolites. Further studies of the reactivity and biomimetic reactions concerning pyrrole-2-aminoimidazolones will be reported in due course.

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Supporting Information Available: Extraction procedure, isolation, and characterization data (including ^{1}H and ^{13}C NMR spectra) for compounds **1** and **2**. Experimental and NMR spectra for synthetic compounds **1** and **2** and for compounds **10**, **13**−**15**, and **17a** + **17b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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