ORGANIC LETTERS

2006
Vol. 8, No. 11
2421−2424

# Verpacamides A–D, a Sequence of $C_{11}N_5$ Diketopiperazines Relating Cyclo(Pro-Pro) to Cyclo(Pro-Arg), from the Marine Sponge *Axinella vaceleti:* Possible Biogenetic Precursors of Pyrrole-2-aminoimidazole Alkaloids

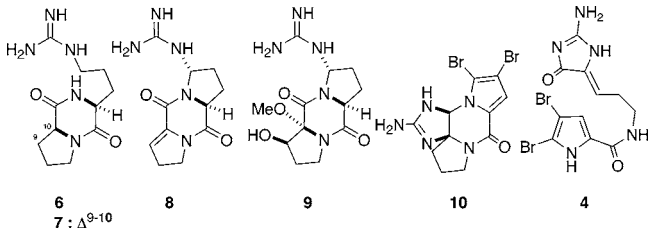
**Carine Vergne,[†] Nicole Boury-Esnault,[‡] Thierry Perez,[‡] Marie-Thérèse Martin,[†] Marie-Thérèse Adeline,[†] Elise Tran Huu Dau,[†] and Ali Al-Mourabit*,[†]**

*Institut de Chimie des Substances Naturelles du CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France, and Centre d'Océanologie de Marseille, CNRS UMR-6540, Station Marine d'Endoume, 13007 Marseille, France*

*ali.almourabit@icsn.cnrs-gif.fr*

**Received April 4, 2006**

## ABSTRACT




**Four $C_{11}N_5$ diketopiperazine metabolites named verpacamides A (6), B (7), C (8), and D (9) consisting of a proline-arginine dipeptide skeleton have been isolated from the marine sponge *Axinella vaceleti.* Verpacamides A–D are a sequence of metabolites showing the transformation of proline and arginine into the oxidized guanidinyl-cyclo(Pro-Pro) 8 and 9. Compounds 6–9 are structurally and chemically related to $C_{11}N_5$ pyrrole-2-aminoimidazole metabolites also isolated from the Axinellidae and Agelasidae families of sponges and exemplified by dispacamide A (4) and dibromophakellin (10).**

The biosynthesis of the pyrrole-2-aminoimidazole (P-2-AI) metabolites isolated from the Agelasidae and Axinellidae families of marine sponges is supposedly dependent on the biosynthetically related amino acids proline and ornithine.[1] As part of our ongoing investigations toward the understanding of the biogenetic chemical pathways involved in this family of alkaloids, we have recently observed that the 2-aminoimidazolone moiety of dispacamide (**4**) could be generated spontaneously from proline and guanidine.[2] As shown in Scheme 1, proline-derived diketopiperazine **1** underwent skeletal spontaneous oxidative rearrangement when exposed to air oxygen and guanidine, yielding dioxetanone **2**[3] which reacted with guanidine to provide ami-

[†] Institut de Chimie des Substances Naturelles du CNRS.
[‡] Centre d'Océanologie de Marseille.

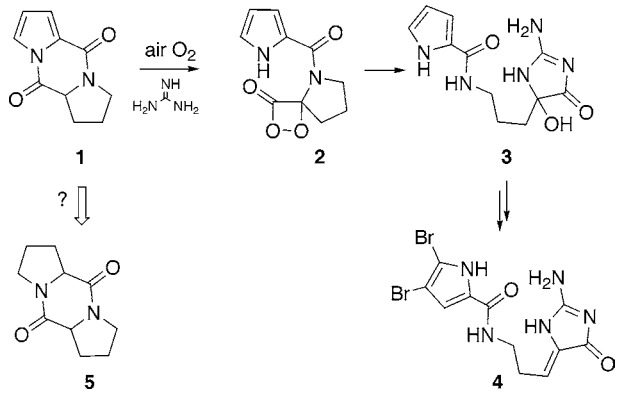
(1) (a) Foley, L. H.; Büchi, G. *J. Am. Chem. Soc.* **1982**, *104*, 1776−1777. (b) Kitagawa, I.; Kobayashi, M.; Kitanaka, K.; Kido, M.; Kyogoku, Y. *Chem. Pharm. Bull.* **1983**, *31*, 2321−2328. (c) Braekman, J.-C.; Daloze, D.; Stoller, C.; Van Soest, R. W. M. *Biochem. Syst. Ecol.* **1992**, *20*, 417−431. (d) Andrade, P.; Willoughby, R.; Pomponi, S. A.; Kerr, R. G. *Tetrahedron Lett.* **1999**, *40*, 4775−4778. (e) Lindel, T.; Hochgürtel, M.; Assmann, M.; Köck, M. *J. Nat. Prod.* **2000**, *63*, 1566−1569.

(2) Travert, N.; Al-Mourabit, A. *J. Am. Chem. Soc.* **2004**, *126*, 10252−10253.

(3) The mechanism of the transformation of **1** into **2**, proposed in ref 6, has been confirmed by the isolation of the dioxetanone intermediate. The mechanistic study will be published elsewhere.

10.1021/ol0608092 CCC: $33.50 
**Published on Web 05/04/2006**

**Scheme 1.** Spontaneous Formation of **3** from **1** in the Presence of Guanidine and Air Oxygen




noimidazolone **3** containing the $C_{11}N_5$ backbone and the oxidation state of dispacamide A (**4**).

This finding suggests that an early precursor such as diketopiperazine cyclo(Pro-Pro) (**5**)[4] combines with guanidine in an oxidative process leading to the formation of the common $C_{11}N_5$ skeleton of the P-2-AI metabolites via a derivative similar to pyrrolic diketopiperazine **1**. The source of the guanidine moiety and the biogenetic pathway by which the guanidine is introduced remain unclear. In an initial biosynthetic study, Kerr et al.[1d] have concluded that arginine, which contains the guanidine motif, is not incorporated into the P-2-AI metabolite stevensine (**11**) (Figure 1). As there

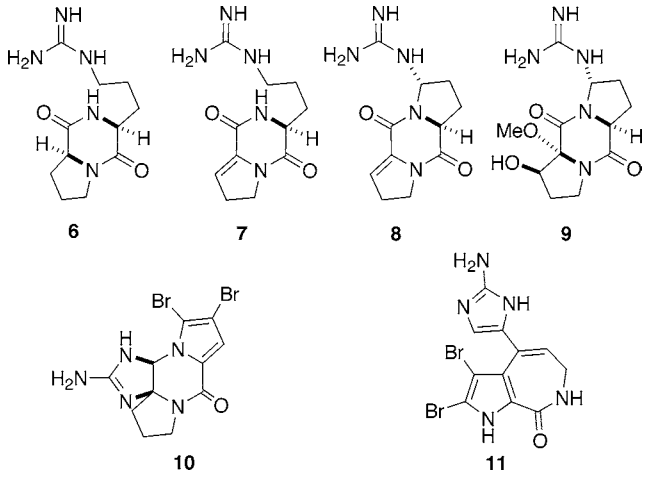



**Figure 1.** Verpacamides A−D (**6**−**9**), dibromophakellin (**10**), and stevensine (**11**).

is no direct biochemical evidence for the structure of the early precursors involved in the formation of the 2-aminoimidazole and 2-aminoimidazolone motifs, hypotheses on the conversion of proline, ornithine, histidine, arginine, or lysine into the P-2-AI metabolites remain purely speculative at this point. Since we made the hypothesis of oxidative proline rearrangement, we directed our attention to an extensive investigation of diketopiperazines containing proline and its oxidized forms in sponges. Our research on the isolation and characterization of new compounds was conducted with special attention to proline derivatives.

After exhaustive exploration of a series of sponge species, our structure-guided investigation led to the discovery, in the sponge *Axinella vaceleti* (Pansini, M. *Boll. Mus. Ist. Biol. Univ. Genova* **1983**, *50*, 79−98), of the interesting tricyclic $C_{11}N_5$ metabolite verpacamide C (**8**) together with its presumed immediate biosynthetic precursors verpacamide A (**6**) and verpacamide B (**7**) (Figure 1). Verpacamide D (**9**), which is an oxidized derivative of **8**, was also isolated. Verpacamide A (**6**) may be derived from proline and arginine and further metabolized into verpacamides B (**7**), C (**8**), and D (**9**).

Two samples of the frozen *Axinella vaceleti*, a Mediterranean sponge collected in April 2003 and July 2004, were subjected to identical extraction procedures. Silica gel chromatography and further HPLC purifications afforded verpacamides A−D (**6**−**9**). Proline-arginine diketopiperazine **6** showed a $^1$H NMR spectrum slightly different from the known natural product isolated from a marine bacterium (*Pseudomonas* sp.), cyclo(L-Arg-D-Pro).[5] Chemical shifts of carbon and proton atoms pointed to the known synthetic cyclo(L-Arg-L-Pro) diastereoisomer which was prepared by us using literature procedures.[6,7] Spectral data of the synthetic and the natural compound **6** were indeed identical. The $^1$H NMR spectra at pH 3 and the optical rotation ($[\alpha]^{24}_D$ = −69.2° ($c$ = 0.4, MeOH)) of our synthetic material were also identical to those reported by Sano et al.[5] This is the first report of the cyclo(L-Arg-L-Pro) stereoisomer as a natural metabolite.

The oxidized diketopiperazine **7** was isolated for complete identification after detection by LCMS analysis. Compound **7** presents a HRMS $(M + H)^+$ ion at $m/z$ 252.1453. $^1$H NMR and $^{13}$C NMR spectra established the molecular formula of $C_{11}H_{17}N_5O_2$ (instead of $C_{11}H_{19}N_5O_2$) for **6**. The C-9/C1-0 unsaturated cyclo(Pro-Arg) structure for **7** was confirmed by two-dimensional NMR analysis. The absolute configuration of the arginine moiety was not established ($[\alpha]^{24}_D$ = −5.7° ($c$ = 0.32, MeOH)). However, the (*S*) configuration of the arginine portion in verpacamide A (**6**) is expected to be the same as that in its derivative **7** that we have named verpacamide B.

LCMS of **8** showed the $(M + H)^+$ ion at $m/z$ 250 and a fragment at $m/z$ 191 (−59). The observation of an isolated carbon atom at 157.3 ppm in DMSO-$d_6$ revealed the presence of a terminal guanidine group. Interpretation of the NMR spectra (1D and 2D) indicated two proline units with one unsaturation. The presence of the diketopiperazine skeleton

(4) (a) Schmidt, U.; Häusler, J. *Angew Chem., Int. Ed. Engl.* **1976**, 497−498. (b) Cyclo (Pro-Pro) **5** has been used by Romo for the total synthesis of the P-2-AI dibromophakellstatin. See :Poullennec, K.; Romo, D. *J. Am. Chem. Soc.* **2003**, *125*, 6344−6345.

(5) Izumida, H.; Imamura, N.; Sano, H. *J. Antibiot.* **1996**, *49*, 76−80.

(6) Kertscher, U.; Bienert, M.; Krause, E.; Sepetov, N. F.; Mehlis, B. *Int. J. Pept. Protein Res.* **1993**, *41*, 207−211.

(7) Boissonnas, R. A.; Guttmann, St.; Jaquenoud, P.-A. *Helv. Chim. Acta* **1960**, *43*, 1349−1355.

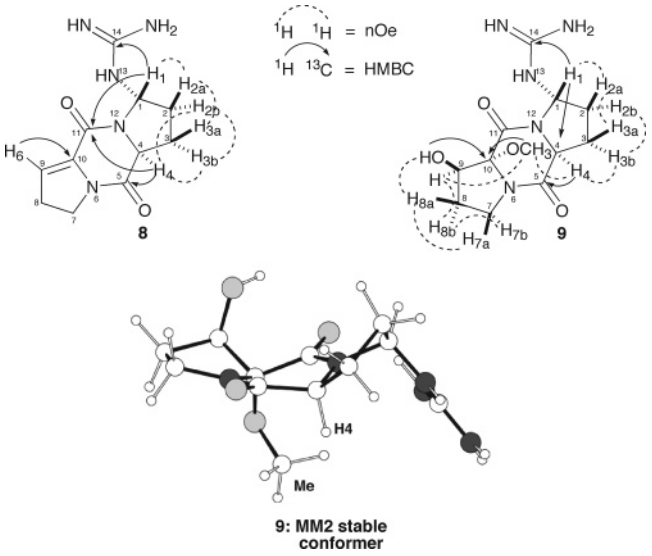



**Figure 2.** Key HMBC/nOe correlations of **8** and **9** and the MM2 energy-minimized representation of **9**.

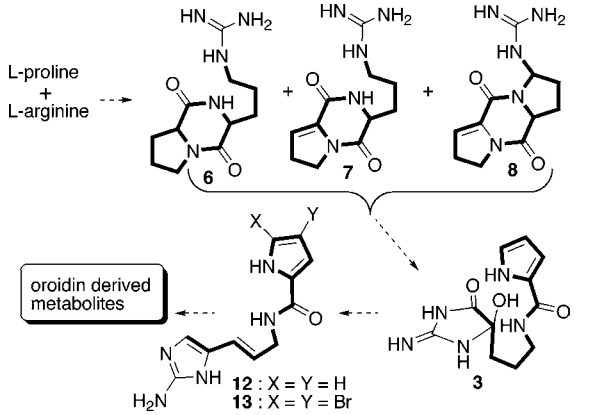
and its linkage to guanidine were established by the HMBC correlation between H-1 and C-14, as shown in Figure 2. The guanidine motif must therefore be attached to the C-1 carbon. Extensive nOe studies proved the anti relative configuration of H-1 and H-4. The absolute configuration of **8**, named verpacamide C, was not established ($[\alpha]^{24}_D$ = +17.4° ($c$ = 0.15, $H_2O$)). Again, the configuration at C-4, derived from arginine, is expected to be (*S*) as in verpacamide A (**6**) and verpacamide B (**7**).

LCMS of the minor product **9**, obtained in only moderate purity, showed the $(M + H)^+$ ion at *m*/*z* 298 and a fragment at 239 (M-59) indicating again the presence of a terminal guanidine group. The NMR spectra of **9** in $CD_3OD$ and in DMSO-$d_6$ revealed the presence of a carbon at 97.0 ppm bearing an $OCH_3$ group and a carbon at 75.1 ppm bearing a OH (exchangeable proton at 5.38 ppm) correlated with a proton at 4.18 ppm. The absence of ethylenic protons, in comparison with **8**, led to the conclusion that the C-9 and C-10 carbons of the double bond in **8** bear OH and OMe groups, respectively, in **9**. This assignment was confirmed by COSY and HMBC experiments. The relative stereochemistry was established by NOESY and ROESY experiments. All NMR correlations were in accordance with the most stable conformer obtained by MM2 energy minimization (Figure 2).

A multitude of diketopiperazine-derived metabolites occurring in nature, including marine organisms[8] and mainly microorganisms,[9] have been isolated. However, there are no examples of proline-proline-guanidine structures such as **8** and **9** reported from marine sponges. Such a natural ring closure of the arginine in **7** into a guanidine-bearing proline functionality in the $C_{11}N_5$ **8** is observed here for the first time. Proline and arginine biosynthetic interconversion was only known via an ornithine intermediate. This putative oxidative cyclization of the arginine part into a proline derivative underscores the relationship among proline, ornithine, and arginine during biogenetic processes.

Taking into account the structures of the newly isolated $C_{11}N_5$ cyclo(Pro-Pro) diketopiperazines **6**−**9**, the model reaction that we have discovered (Scheme 1), and the fact that these metabolites were also identified in the Axinellidae sponge family, we consider that the significance of these findings may be important for the search of the early biosynthetic precursors of pyrrole-2-aminoimidazole metabolites. Indeed, $C_{11}N_5$ dispacamide A (**4**) (Scheme 1) and dibromophakellin (**10**) (Figure 1) belong to the pyrrole-2-aminoimidazole family that contains nearly 100 members isolated from phylogenetically related sponges. The biomimetic intermolecular easy oxidative reaction between the tricycle **1** and guanidine (Scheme 1) could naturally take place in an intramolecular manner starting from a derivative of **6**, **7,** or **8** (Scheme 2). These diketopiperazines can be

**Scheme 2.** Proposed Early Precursors for $C_{11}N_5$ Metabolites

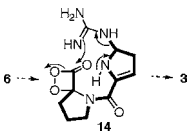



considered as a combination of proline and arginine or proline-proline and guanidine. Verpacamides B (**7**) and C (**8**) may be considered as the first oxidized members of the pathway transforming the proline skeleton into pyrrole and 2-aminoimidazolinone derivatives. The supported cascade begins with the generation of the tricyclic structure from $C_{11}N_5$ cyclo(Pro-Arg) **6** and its further oxidation into a

(8) (a) Schmidtz, F. J.; Vanderah, D. J.; Hollenbeak, K. H.; Enwall, C. E. L.; Gopichand, Y.; SenGupta, P. K.; Hossain, M. B.; Van der Helm, D. *J. Org. Chem.* **1983**, *48*, 3941−3945. (b) Adamczeski, M.; Quinoa, E.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 647−654. (c) Sölter, S.; Dieckmann, R.; Blumenberg, M.; Francke, W. *Tetrahedron Lett.* **2002**, *43*, 3385−3386.

(9) (a) Shigemori, H.; Tenma, M.; Shimazaki, K.; Kobayashi, J. *J. Nat. Prod.* **1998**, *61*, 696−698. (b) Macherla, V. R.; Liu, J.; Bellows, C.; Teisan, S.; Nicholson, B.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2005**, *68*, 780−783. (c) Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 1226−1231. (d) Fadhila, F.; Vasquez, V.; Sanchez, J.-L.; Riguera, R. *J. Nat. Prod*, **2003**, *66*, 1299−1301.

(10) Possible transformation of **6** into **3** via a dioxetanone intermediate **14**:



dioxetanone derivative such as **14**.[10] The driving force of the conversion into pyrrole-2-aminoimidazolinone derivative **3** would be the aromatization of one proline into pyrrole and the intramolecular transfer of guanidine.

The oxidation and rearrangement of the diketopiperazine **6** could be involved in a chemical pathway leading to the $C_{11}N_5$ dispacamide A (**4**) and then to the central precursors clathrodine (**12**) and oroidin (**13**). The latter intermediates play an important role in the production of the very challenging P-2-AI metabolites.[11]

The function of the proposed chemical pathway in the detoxification and production of defense metabolites in marine sponges is an extremely interesting ecological matter.[12]

The possible participation of microorganism symbionts of sponges in the production of these diketopiperazines is an interesting question. It is frequent that secondary metabolites, in particular, fungal metabolites, derive from two amino acids by way of their dipeptide form.

In conclusion, verpacamides A−D (**6**−**9**) showing the unprecedented natural oxidative cyclization of the cyclo(Pro-Arg) into 1-guanidino-cyclo(Pro-Pro) metabolites were isolated. To the best of our knowledge, this is the first report of a cyclized arginine into a guanidine-bearing proline. The easy oxidative rearrangement discovered in our group could occur in nature through an intramolecular transfer of guanidine via a dioxetanone intermediate. Further investigations toward the exhaustive isolation of additional proline and arginine precursors from sponges and synthetic studies are underway.

**Acknowledgment.** We gratefully acknowledge Dr. Robert Dodd for the improvements to this manuscript.

**Supporting Information Available:** Extraction procedures and isolation and characterization data (including $^1$H and $^{13}$C NMR spectra) for compounds **6**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0608092

(11) For further developments of the biomimetic reactivity of 2-aminoimidazole derivatives see: (a) Al-Mourabit, A.; Potier, P. *Eur. J. Org. Chem.* **2001**, 237−243. (b) Abou-Jneid, R.; Ghoulami, S.; Martin, M.-T.; Tran Huu Dau, E.; Travert, N.; Al-Mourabit, A. *Org. Lett.* **2004**, *6*, 3933−3936. (c) Baran, P. S.; O'Malley, D. P.; Zografos, A. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 2674−2677.

(12) Rees, J.-F.; De Wergifosse, B.; Noiset, O.; Dubuisson, M.; Janssens, B.; Thompson, E. M. *J. Exp. Biol*. **1998**, *201*, 1211−1221.