ORGANIC LETTERS

2010
Vol. 12, No. 22
5158−5161

Heronapyrroles A−C: Farnesylated 2-Nitropyrroles from an Australian Marine-Derived *Streptomyces* sp.

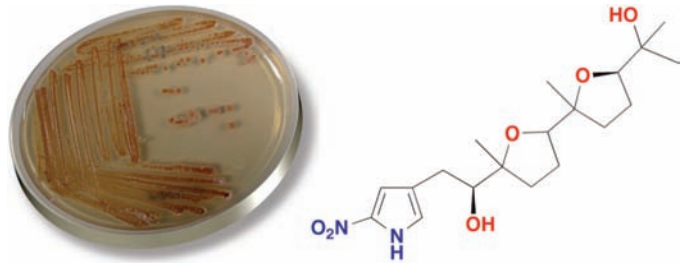
Ritesh Raju, Andrew M. Piggott, Leticia X. Barrientos Diaz, Zeinab Khalil, and Robert. J. Capon*

Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia

r.capon@uq.edu.au

Received September 10, 2010

ABSTRACT



Chemical analysis of a marine-derived *Streptomyces* sp. (CMB-M0423) isolated from beach sand off Heron Island, Australia, yielded three new members of the rare pyrroloterpene biosynthetic structure class. Identified by detailed spectroscopic analysis as the first reported examples of naturally occurring 2-nitropyrroles, heronapyrroles A−C (1−3) displayed promising biological activity—with low to submicromolar IC_{50} activity against Gram-positive bacteria but no cytotoxicity toward mammalian cell lines.

During our investigations into the secondary metabolism of Australian marine-derived microbes, we recovered a *Streptomyces* sp. (CMB-M0423) from a shallow water (−1 m) sand sample obtained off Heron Island, Queensland. HPLC-DAD-MS analysis of a small-scale saline liquid cultivation (100 mL) revealed biosynthetically related yellow pigments [*m*/*z* 421 (**1**), 405 (**2**), and 407 (**3**)] sharing an unusual polyene-like chromophore (λ_{max} = 348). Related pigments were also evident in saline solid-phase cultures but were absent from nonsaline liquid and solid cultures. HPLC fractionation of a larger scale saline liquid culture (3 L) permitted recovery of **1**−**3**, which spectroscopic analysis revealed as rare mixed biosynthesis pyrroloterpenes and the first reported examples of natural products bearing a 2-nitropyrrole. An account of the spectroscopic analyses leading to assignment of structures to heronapyrroles A−C (**1**−**3**) (Figure 1) and discussion on a plausible biosynthesis and biological properties are presented below.

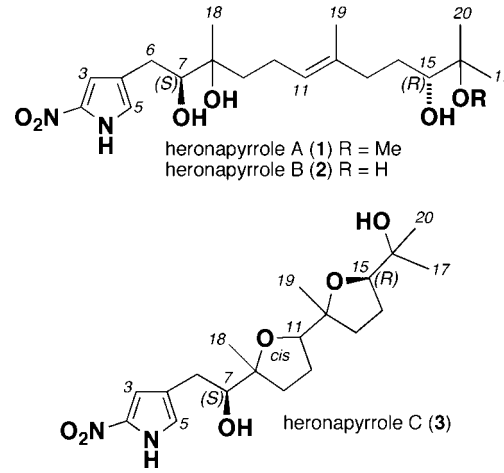


Figure 1. Heronapyrroles A−C (**1**−**3**).

10.1021/ol102162d
Published on Web 10/15/2010

Table 1. Comparative ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR (MeOH-d_4) Data for Heronapyrroles A−C (**1**−**3**)

	heronapyrrole A (**1**)		heronapyrrole B (**2**)		heronapyrrrole C (**3**)	
pos.	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C	δ_H, mult (*J* in Hz)	δ_C
2		138.2		138.6		138.3
3	7.03, d (*1.8*)	112.1	7.03, s	112.1	7.00, d (*1.7*)	111.9
4		125.8		126.2		125.7
5	6.93, d (*1.8*)	124.3	6.93, s	124.1	6.93, d (*1.7*)	124.2
6a	2.86, dd (*14.8*, *1.9*)	29.3	2.86, d (*14.8*)	29.5	2.82, dd (*14.8*, *1.9*)	29.9
6b	2.44, dd (*14.8*, *10.7*)		2.43, dd (*14.8*, *10.7*)		2.44, dd (*14.8*, *10.4*)	
7	3.46, dd (*10.7*, *1.9*)	79.0	3.46, d (*10.7*)	79.1	3.59, dd (*10.4*, *1.9*)	78.6
8		74.9		75.2		86.8
9a	1.58, m	39.7	1.58, m	39.8	2.15, ddd (*12.8*, *8.9*, *4.3*)	35.0
9b	1.58, m		1.58, m		1.67, m	
10a	2.14, m	22.5	2.14, m	22.7	1.97[a], m	28.2
10b	2.14, m		2.14, m		1.82, m	
11	5.22, dd (*8.3*, *7.3*)	125.8	5.22, t (*6.9*)	126.0	4.02, dd (*8.6*, *6.5*)	85.9
12		135.6		135.9		86.0
13a	2.24, ddd (*13.9*, *9.9*, *4.5*)	37.5	2.25, ddd (*14.2*, *8.4*, *4.4*)	37.8	1.95[a], m	34.6
13b	2.01, ddd (*13.9*, *9.4*, *7.5*)		2.02, m		1.63, m	
14a	1.67, m	30.3	1.72, m	30.7	1.88, m	27.3
14b	1.34, m		1.35, m		1.88, m	
15	3.37, dd (*10.5*, *1.7*)	76.5	3.24, d (*10.7*)	78.9	3.81, dd (*8.3*, *7.1*)	88.8
16		78.3		73.5		72.0
17	1.10, s	20.2	1.16, s	25.5	1.17, s	26.0
18	1.17, s	21.1	1.17, s	21.4	1.22, s	22.0
19	1.65, s	15.8	1.65, s	15.9	1.21, s	25.0
20	1.14, s	20.8	1.12, s	24.6	1.13, s	25.8
16-OMe	3.21, s	49.2				

[a] Overlapping signals. ^{13}C shifts obtained from 2D HSQC and HMBC experiments.

HRESI(+)MS analysis of **1** revealed a pseudomolecular ion ($[M + Na]^+$) indicative of a molecular formula ($C_{20}H_{34}N_2O_6$, Δmmu 0.1) requiring five double bond equivalents (DBEs). The NMR (MeOH-d_4) data for **1** (Table 1) revealed two tertiary methyls (δ_H 1.10, H_3-17; 1.14, H_3-20) and an *O*-methyl (δ_H 3.21, H_3-20) linked by HMBC correlations to quaternary (δ_C 78.3, C-16) and tertiary (δ_C 76.5, C-15) oxy carbons, with COSY correlations extending connectivity from H-15 to the methylenes H_2-14 and H_2-13 (Figure 2, subunit C-13 to C-17 inclusive of C-20). Further

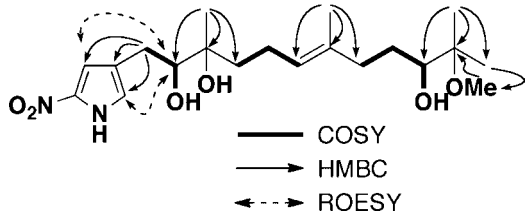


Figure 2. Key 2D NMR correlations for heronapyrrole A (**1**).

NMR analysis revealed an olefinic methyl (δ_H 1.65, H_3-19) with HMBC correlations to C-13 and an olefinic methine carbon (δ_C 125.8, C-11), with COSY correlations extending connectivity from H-11 to the methylenes H_2-10 and H_2-9. HMBC correlations established the remaining tertiary methyl (δ_H 1.17, H_3-18) as a pendant to an oxy quaternary carbon (δ_C 74.9, C-8) flanked by C-9 and C-7, with COSY correlations extending connectivity from H-7 to the methylene H_2-6. Analysis of the NMR (DMSO-d_6) data for **1** (Supporting Information, Figure S1b and Table S1b) revealed resonances and correlations consistent with 7-OH (δ_H 4.53), 8-OH (δ_H 4.08), and 15-OH (δ_H 4.37). The observations outlined above (summarized in Figure 2) confirm the C-6 to C-20 structure subunit as shown, leaving $C_4H_3N_2O_2$ and four DBEs unaccounted.

Further analysis of the NMR (MeOH-d_4) data for **1** revealed two deshielded sp^2 methines (δ_H 7.03, H-3; 6.93, H-5) with HMBC correlations across four sp^2 carbons (δ_C 138.2, C-2; 112.1, C-3; 125.8, C-4; 124.3, C-5) necessitating that the remaining proton be exchangeable and suggestive of a pyrrole. ^{13}C NMR shifts for the pyrrole carbons precluded direct substitution by oxygen, pointing instead to a disubstituted nitropyrrole. ROESY (DMSO-d_6) correlations from both H-3 and H-5 to H_2-6 supported the pyrrole regiochemistry as indicated. Consistent with this proposal, a literature account[1] of synthetic nitropyrroles provided diagnostic UV−vis maxima for 2-nitropyrrole (335 nm) versus 3-nitropyrrole (268 nm), in accord with the proposed 2-nitropyrrole regiochemistry for **1** (348 nm).

(1) Koyama, M.; Kodama, Y.; Tsuruoka, T.; Ezaki, N.; Niwa, T.; Inouye, S. *J. Antibiot.* **1981**, *34*, 1569.

HRESI(+)MS analysis of **2** revealed a pseudomolecular ion ($[M + Na]^+$) indicative of a molecular formula ($C_{19}H_{32}N_2O_6$, Δmmu -0.7) consistent with a homologue ($-CH_2$) of **1**. As the only significant difference in the NMR data for **2** (Table 1, and Supporting Information, Figure S2a and Table S2a) compared to **1** was replacement of resonances for the 16-OMe with a 16-OH, **2** was determined to be an O-demethylated analogue of **1**.

HRESI(+)MS analysis of **3** revealed a pseudomolecular ion ($[M + Na]^+$) indicative of a molecular formula ($C_{19}H_{30}N_2O_6$, Δmmu $+0.1$) requiring six DBEs. Comparison of the UV−vis and NMR data for **3** with those for **1** and **2** (Table 1, and Supporting Information, Figure S3a and Table S3a) confirmed the presence of a common 2-nitropyrrole terminus with a C-4 oxidized farnesyl substituent. Whereas the farnesyl side chain in **1** and **2** possessed a common *E* $\Delta^{11,12}$ double bond, this moiety in **3** was oxygenated, contributing to a suite of tertiary (C-7, C-11, and C-15) and quaternary (C-8, C-12, and C-16) sp^3 oxycarbon resonances. Analysis of the 2D NMR (MeOH-d_4) data for **3** established HMBC connectivity between four oxy tertiary methyls (H_3-17, H_3-18, H_3-19, and H_3-20) and three separate COSY correlated spin systems (H_2-6 to H-7, H_2-9 to H-11, and H_2-13 to H-15) and from H_2-6 to the 2-nitropyrrole carbons (C-3 to C-5)—defining the overall carbon skeleton and heteroatom regiochemistry (Figure 3). With two of the six available

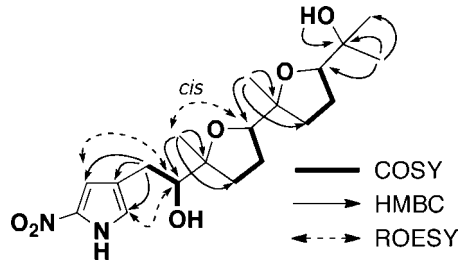


Figure 3. Key 2D NMR correlations for heronapyrrole C (**3**).

oxygen atoms, and four of the six DBEs in **3** attributed to the 2-nitropyrrole moiety, and with the NMR (DMSO-d_6) data for **3** (Supporting Information, Figure S3b and Table S3b) confirming the presence of 7-OH (δ_H 4.69) and 16-OH (δ_H 3.95) moieties, it was determined that **3** possessed two cyclic ether residues spanning C-8, C-11, C-12, and C-15. A ROESY (DMSO-d_6) correlation from H-11 to H_3-18 positioned these ethers as two tetrahydrofuran rings spanning C-8 to C-11, and C-12 to C-15, with the former possessing a *cis* relative configuration as shown.

To assign absolute configurations at C-7 and C-15 in **1**, we prepared the bis-(*R*)-MTPA and bis-(*S*)-MTPA esters as part of a Mosher analysis.[2] The ^1H NMR ($CDCl_3$) spectra of these esters (Supporting Information, Figures S5 and S6) revealed a negative $\Delta\delta_{S-R}$ for H-6a (-0.10) and H-6b (-0.15) and a positive $\Delta\delta_{S-R}$ for H-14a ($+0.06$) and H-14b ($+0.06$), confirming absolute configurations of 7*S* and 15*R*, respectively. Unfortunately, low yields of **1**−**3** from microbial culture precluded further chemical derivatization or degradative approaches to the assignment of remaining stereocenters. However, we tentatively infer 7*S* and 15*R* absolute configurations for **2** and **3** on biosynthetic grounds.

The heronapyrroles A−C (**1**−**3**) belong to a rare mixed pyrroloterpene structure class, known examples of which are limited to the lipid peroxidation inhibitor pyrrolostatin from a Brazilian soil *Streptomyces chrestomyceticus*[4] and the antimicrobial and anticancer glaciapyrroles A−C from an Alaskan marine-derived *Streptomyces* sp.[5,6] A plausible biosynthesis for the heronapyrroles is outlined in Figure 4.

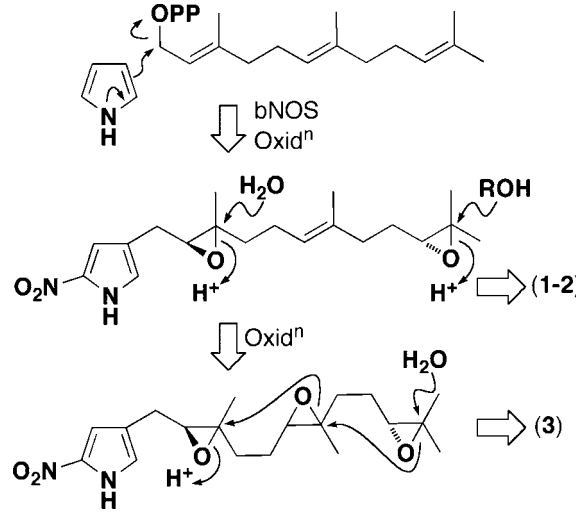


Figure 4. Plausible biosynthesis of heronapyrroles.

In this pathway, aromatic substitution of pyrrole by farnesyl pyrophosphate provides ready access to the pyrroloterpene carbon framework which, following nitration, oxidation, and nucleophilic ring opening of epoxide intermediates, with and without cascade cyclization to polyethers, is capable of elaborating the full suite of heronapyrroles. With respect to the nitro functionality, we draw attention to a 2004 report,[3] which describes bacterial nitric oxide synthase-initiated nitration of secondary metabolites.

Heronapyrroles are also noteworthy in that they belong to the exceptionally rare family of nitropyrrole natural products, known examples of which are limited to the 3-nitropyrrole pyrrolomycin class of *Streptomyces* antibiotics.[1,7−9] Furthermore, the heronapyrroles are the first documented examples of natural products bearing a 2-nitropyrrole functionality.[10]

(2) Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451.

(3) Kers, J. A.; Wach, M. J.; Krasnoff, S. B.; Widom, J.; Cameron, K. D.; Bukhalid, R. A.; Gibson, D. M.; Crane, B. R.; Loria, R. *Nature* **2004**, *429*, 79.

(4) Kato, S.; Shindo, K.; Kawai, H.; Odagawa, A.; Matsuoka, M.; Mochizuki, J. *J. Antibiot.* **1993**, *46*, 892.

(5) Macherla, V. R.; Liu, J. N.; Bellows, C.; Teisan, S.; Nicholson, B.; Lam, K. S.; Potts, B. C. M. *J. Nat. Prod.* **2005**, *68*, 780.

(6) Macherla, R.; Rami, V. United States Patent 20060052436.

(7) Ezaki, N.; Shomura, T.; Koyama, M.; Niwa, T.; Kojima, M.; Inouye, S.; Ito, T.; Niida, T. *J. Antibiot.* **1981**, *34*, 1363.

Significantly, although the heronapyrroles displayed no cytotoxicity (IC_{50} > 30 μM) when tested against human cervical carcinoma (HeLa), colorectal carcinoma (HT-29), and gastric adenocarcinoma (AGS) cell lines and were inactive (IC_{50} > 50 μM) against the Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 11775, they did display very promising activity against the Gram-positive bacteria *Staphylococcus aureus* ATCC 9144 (IC_{50} 0.6−1.1 μM) and *Bacillus subtilis* ATCC 6633 (IC_{50} 1.1−6.5 μM) (Supporting Information, Table S4).

In summary, the heronapyrroles are the first reported examples of a novel class of secondary metabolite, farnesylated 2-nitropyrroles, and exhibit promising Gram-positive selective antibacterial properties with no mammalian cytotoxicity.

Acknowledgment. We thank M. Gauthier (UQ) for the collection of sand samples and M. Conte (UQ) for cytotoxicity screening. R.R. acknowledges the provision of an Australian Postgraduate Award and Z.K. the provision of a UQ International Postgraduate Student Award. L.B. acknowledges the financial aid provided by the Attraction and Insertion program of CONICYT (PSD32), Chile, and the Performance Agreement (Decreto No. 002251/07) signed between the Ministry of Education of Chile and the Universidad de La Frontera. This research was funded in part by the Institute for Molecular Bioscience, The University of Queensland, and the Australian Research Council (LP0989954).

Supporting Information Available: Details of collection, extraction, cultivation, and taxonomy of the microorganism. Full characterization of all compounds as well as ^{1}H NMR spectra and tabulated 1D and 2D NMR data for all compounds in MeOH-d_4 and DMSO-d_6. This material is available free of charge via the Internet at http://pubs.acs.org.

OL102162D

(8) Carter, G. T.; Nietsche, J. A.; Goodman, J. J.; Torrey, M. J.; Dunne, T. S.; Borders, D. B.; Testa, R. T. *J. Antibiot.* **1987**, *40*, 233.

(9) Charan, R. D.; Schlingmann, G.; Bernan, V. S.; Feng, X. D.; Carter, G. T. *J. Nat. Prod.* **2005**, *68*, 277.

(10) The existence of natural farnesylated 2-nitropyrroles has been foreshadowed in the patent (Kuzuyama, T.; Noel, J. P.; Richard, S. P. United States Patent 200600316) and review literature. Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666. However, such molecules have not been documented until now.