ORGANIC LETTERS

XXXX
Vol. xx, No. x

Naseseazines A and B: A New Dimeric Diketopiperazine Framework from a Marine-Derived Actinomycete, *Streptomyces* sp.

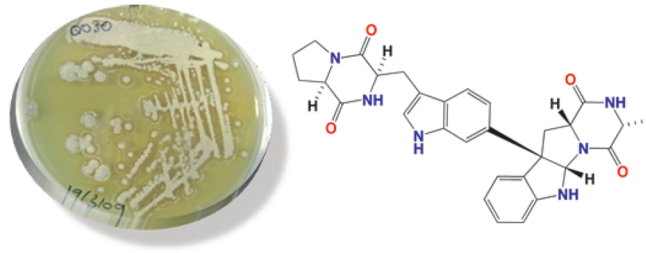
Ritesh Raju,[†] Andrew M. Piggott,[†] Melissa Conte,[†] William G. L. Aalbersberg,[‡] Klaus Feussner,[‡] and Robert J. Capon*,[†]

Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland 4072, Australia, and Institute of Applied Science, The University of the South Pacific, Suva, Fiji

r.capon@uq.edu.au

Received June 29, 2009

ABSTRACT



Chemical analysis of a *Streptomyces* sp. (CMB-MQ030) isolated from a Fijian marine sediment yielded two new diketopiperazines, naseseazines A and B (1, 2), featuring a new dimeric framework. Structures were determined by detailed spectroscopic analysis and C_3 Marfey's analysis.

The 2,5-diketopiperazine (DKP) structure class is represented in a wide array of structurally diverse natural products, with many examples displaying potent and selective biological properties, including the ability to modulate key pathways in cardiovascular and infectious diseases, cancer, pain and other therapeutic indications.[1] Within the field of cancer, for example, bioactive DKPs range across the phenylahistins (antimicrotubule),[2] tryprostatins (cell cycle inhibitors),[3] chaetocins (lysine-specific histone methyltransferase inhibitors)[4] and ardeemins (inhibitors of multiple drug resistant phenotypes),[5] to list but a few. Natural DKPs are cyclic dipeptides biosynthetically assembled by either nonribosomal peptide synthetases (NRPSs) or the more recently discovered cyclodipeptide synthases (CDPs),[6] with structural diversity achieved through choice of amino acid precursors, and subsequent formation of heterocycles, prenylation, oxidation, dimerizations etc.[1] Despite their inherent structural simplicity, DKPs feature a conformationally restrained heterocyclic core that is resistant to proteolysis and capable of providing favorable pharmacodynamic and pharmacokinetic characteristics. The rich heritage of bioactive natural DKPs, particularly those derived from microbial biodiversity, has defined a privileged

[†] The University of Queensland.
[‡] The University of the South Pacific.

(1) Martins, M.; Carvalho, I. *Tetrahedron* **2007**, *63*, 9923.
(2) Kanoh, K.; Kohno, S.; Katada, J.; Takahashi, J.; Uno, I.; Hayashi, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1451.
(3) Cui, C.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 527.
(4) Greiner, D.; Bonaldi, T.; Eskeland, R.; Roemer, E.; Imhof, A. *Nat. Chem. Biol.* **2005**, *1*, 143.
(5) Chou, T.; Depew, K.; Zheng, Y.; Safer, M.; Chan, D.; Helfrich, B.; Zatorska, D.; Zatorski, A.; Bornmann, W.; Denishefsky, S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8369.
(6) Gondry, M.; Sauguet, L.; Belin, P.; Thai, R.; Amourous, R.; Telier, C.; Tuphile, K.; Jacquet, M.; Braud, S.; Courcon, M.; Masson, C.; Dubois, S.; Lautru, S.; Lecoq, A.; Hashimoto, S.; Genet, R.; Pernodet, J. *Nat. Chem. Biol.* **2009**, *5*, 414.

10.1021/ol901466r CCC: $40.75

Table 1. NMR (600 MHz, methanol-d_4) Data for Naseseazine A (**1**)

position	δ_H, mult, (*J* in Hz)	δ_C	COSY	$^1H-^{13}C$ HMBC	ROESY
2	5.83, s	87.1		9, 11, 12, 7′	11, 8′, 6′
3		61.2			
4		135.8			
5	6.85, d, (*7.4*)	124.9	6	3, 7, 9	12a/b
6	6.67, t, (*7.5*)	120.2	5, 7	4, 8	
7	7.05, t, (*7.2*)	129.2	6, 8	5, 9	
8	6.69, d, (*7.6*)	110.9	7	4, 6	
9		149.1			
11	4.64, dd, (*8.4*, *7.4*)	60.2	12a/b	12, 13	2, 12b, 15, 8′
12a	2.59, dd, (*13.7*, *10.2*)	39.7	11, 12b	4, 11, 13, 7′	
12b	3.26, m		12a	2, 3, 7′	11, 8′, 6′
13		172.6			
15	4.15, q, (*6.9*)	52.1	17	16, 17	11
16		170.6			
17	1.38, d, (*6.9*)	15.2	15	15, 16	
2′	7.11, s	126.2		3′, 4′, 9′	
3′		109.5			
4′		127.6			
5′	7.57, d, (*8.4*)	120.3	6′	3′, 7′, 9′	11′, 12′b
6′	7.02, d, (*8.4*)	119.5	5′	3, 4′, 8′	2, 11, 12b
7′		137.2			
8′	7.40, s	110.1		3, 4′, 6′	
9′		137.9			
11′	4.39, br t, (*4.5*)	57.2	12′a/b	3′, 12′, 13′, 16′	12′b
12′a	3.28, m	29.0	11′	2′, 3′, 4′, 11′, 13′	
12′b	3.30, m		11′		
13′		167.3			
15′	3.97, dd, (*10.8*, *6.6*)	60.0	17′a/b, 18′a	16′	18′b
16′		170.6			
17′a	0.93, m	29.0	15′, 17′b, 18′a/b	15′, 16′, 18′	
17′b	1.97, m		15′, 17′a, 18′a/b	15′, 18′, 19′	
18′a	1.43, m	22.5	15′, 17′a, 18′b, 19′a/b		
18′b	1.66, m		18′a, 19′a/b	15′	
19′a	3.24, m	45.8	18′a/b, 19′b	17′, 18′	
19′b	3.42, dt, (*11.8*, *8.1*)		18′a/b, 19′a	13′, 15′, 17′, 18′	

structural motif that is an attractive target for the rational development of new therapeutic agents.[1] Alert to the potential of DKPs, new natural product exemplars of this structure class provide valuable inspiration to further expand the repertoire of DKP analogues employed in drug discovery. This report describes the discovery, isolation and structure elucidation of unprecedented DKP dimers, the naseseazines, from a Fijian marine-sediment derived actinomycete.

A marine broth (3 L) fermentation of *Streptomyces* sp. (CMB-MQ030) was extracted with EtOAc, triturated with a range of solvents, and subjected to reverse phase HPLC to yield two dimeric DKPs, naseseazines A (**1**) and B (**2**).

Naseseazine A (**1**) was assigned a molecular formula ($C_{30}H_{30}N_6O_4$) on the basis of HR(+)ESIMS analysis ($[M+Na]^+$ Δmmu −0.5), that required 19 double bond equivalents (DBE). Examination of the NMR (methanol-d_4) data for **1** (Table 1) revealed eighteen sp^2 carbon resonances, fourteen of which were attributed to aromatic (δ_C 109.5 to 149.1) and four to amide carbonyl (δ_C 167.3 to 172.6) carbons, accounting for 11 DBE, and requiring that **1** be octacyclic. Further analysis of the 1D and 2D NMR data suggested the presence of two DKP subunits. Subunit A was identified as a pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole-1,4-dione heterocyclic system based on a *cyclo*-(Ala-Trp) core. The ^1H and COSY NMR data for subunit A (C-1 to C-17) revealed four isolated spin systems; (i) Trp aromatic protons (H-5, H-6, H-7 and H-8), (ii) Trp α and β protons (H-11 and H_2-12), (iii) Ala α and β protons (H-15 and H_3-17), and (iv) a deshielded pyrrolo[2,3-*b*]indole methine (H-2). HMBC correlations from the α-protons identified the Trp (C-13) and Ala (C-16) amide carbonyls respectively, while key HMBC correlations (H-2 to C-9, C-11 and H-12; H-5 to C-3; and H_2-12 to C-4) defined the pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole-1,4-dione heterocycle. The absence of coupling to H-2 (δ_H 5.83, s) and a quaternary C-3 (δ_C 61.2) suggested a C-3 linkage between subunit A and B. This was supported by diagnostic HMBC correlations between H-8′ to C-3, H-12a to C-7′ and H-2 to C-7′. The NMR data for subunit B (C-1′ to C-19′) was consistent with a C-19 substituted *cyclo*-(Pro-Trp). In particular, a sequence of 2D NMR correlations from C-1′ to C-13′ was attributed to a Trp residue, while a sequence from C-15′ to C-19′ was attributed to a Pro residue. HMBC correlations from the Trp α-proton (H-11′) to the Pro amide carbonyl (C-16′), and from the Pro amido-methylene (H_2-19′) to the Trp amide carbonyl (C-13′), defined the DKP moiety.

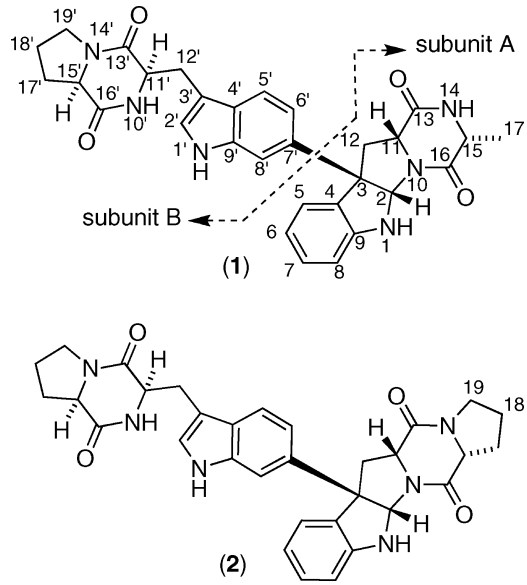


Figure 1. Naseseazines A (**1**) and B (**2**).

The presence of a C-7′ substituent to subunit B was established by analysis of the ^{1}H NMR resonances for H-8′ (δ_H 7.40, s) and H-6′ (δ_H 7.02, d, J = 8.4 Hz) which, together with arguments presented above, established the complete planar structure for naseseazine A (**1**) as shown.

A C_3 Marfey's analysis[7] of **1** confirmed the presence of D-Ala and D-Pro residues, thereby assigning a 15*R*,15′*R* configuration. Diagnostic ROESY correlations across H-2, H-11, H-15, H-6′, and H-8′ positioned these protons on a common β-face of subunit A, defining a 2*S*,3*R*,11*R*,15*R* configuration (Figure 2). Assignment of the configuration about C-11′ proved more problematic, as has been noted in earlier studies into related dimeric DKPs.[8,9]

A comparison of literature ^{1}H NMR (DMSO-d_6) measurements for the α-Trp and α-Pro resonances in natural (α-Trp δ_H 4.29, dd, J = 4.8 and 5.7 Hz; α-Pro δ_H 4.05)[10] and synthetic (α-Trp δ_H 4.30, dd, J = 5.5 and 5.5 Hz; α-Pro δ_H 4.06, dd, J = 8.0 and 8.6 Hz)[11] brevianamide F [also known as *cyclo*-(L-Pro-L-Trp)] with those measured for **1** (α-Trp δ_H 4.28, dd, J = 5.0 and 5.0 Hz; α-Pro δ_H 4.07, dd, J = 8.4 and 8.4 Hz) (see Supporting Information, Table S1) suggested a common relative configuration, consistent with an 11′*R*,15′*R* configuration. This stereochemical assignment was confirmed by ROESY (DMSO-d_6) correlations between H-11′ and H-15′. The absolute configuration for naseseazine A (**1**) could therefore be assigned as indicated, being biosynthetically assembled from all D-amino acids.

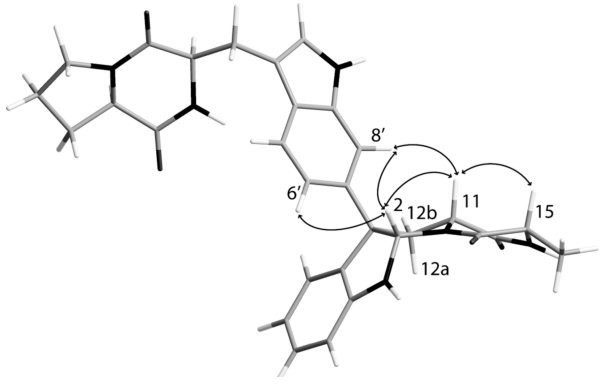


Figure 2. Energy-minimized (MM2) structure of **1** showing key ROESY correlations in subunit A.

Naseseazine B (**2**) was assigned a molecular formula ($C_{32}H_{32}N_6O_4$) on the basis of HR(+)ESIMS analysis ([M + Na]$^+$ Δ mmu −0.6) that, on consideration of the NMR data, was suggestive of a close structure analogue of **1**. More specifically, **1** and **2** possessed an identical subunit B, whereas subunit A in **2** differed from that in **1** by replacement of D-Ala with a D-Pro residue, as confirmed by C_3 Marfey's analysis. A full 1D and 2D NMR (DMSO-d_6 and methanol-d_4) analysis of **2** (Supporting Information, Tables S2 and S3) revealed diagnostic correlations as detailed above for **1**, which defined the structure for **2**, with complete absolute configuration, as indicated.

The naseseazines are members of an extremely rare class of dimeric diketopiperazines featuring a linkage between C-3 of subunit A and the Trp aromatic ring of subunit B. Known natural analogues are limited to two fungal metabolites, asperazine and pestalazine A. Asperazine was reported in 1997 from a sponge-derived *Aspergillus niger* and was noted for lacking antibacterial or antifungal activity, but displaying potent and selective cytotoxicity against a mouse leukemia (L1210) cell line.[8] Pestalazine A was reported in 2008 from the plant pathogenic fungus *Pestalotiopsis theae*, was also noted for a lack of antifungal activity, and was observed to inhibit HIV-1 replication in C8166 cells (EC_{50} 47.6 μM).[9] As new members to this rare class of dimeric diketopiperazines, the naseseazines display added novelty in that they (a) are bacterial not fungal in origin, (b) incorporate only D-amino acid residues, and (c) possess a unique carbon framework defined by a C-3 to C-7′ linkage. A plausible biosynthetic pathway leading to dimerization of DKPs with associated heterocycle formation is outlined in Figure 3. This hypothesis draws on differing indole resonance structures to drive regioselectivity, leading to formation of the naseseazines (pathway A), the pestalazines (pathways B and C), and asperazine (pathway C). The naseseazines, pestalazines, and asperazine are currently the only known natural examples of such dimeric DKPs.

The naseseazines were determined to be noncytotoxic in antibacterial and antifungal assays against *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans*, respectively, and also proved to

(7) Ratnayake, R.; Fremlin, L.; Lacey, E.; Gill, J.; Capon, R. *J. Nat. Prod.* **2008**, *71*, 403.
(8) Varoglu, M.; Corbett, T.; Valeriote, F.; Crews, P. *J. Org. Chem.* **1997**, *62*, 7078.
(9) Ding, G.; Jiang, L.; Guo, L.; Chen, X.; Zhang, H.; Che, Y. *J. Nat. Prod.* **2008**, *71*, 1861.
(10) Zhang, D.; Noviendri, D.; Nursid, M.; Yang, X.; Son, B. *Nat. Prod. Sci.* **2007**, *13*, 251.
(11) Grant, G.; Hunt, A.; Milne, P.; Roos, H.; Joubert, J. *J. Chem. Crystallogr.* **1999**, *29*, 435.

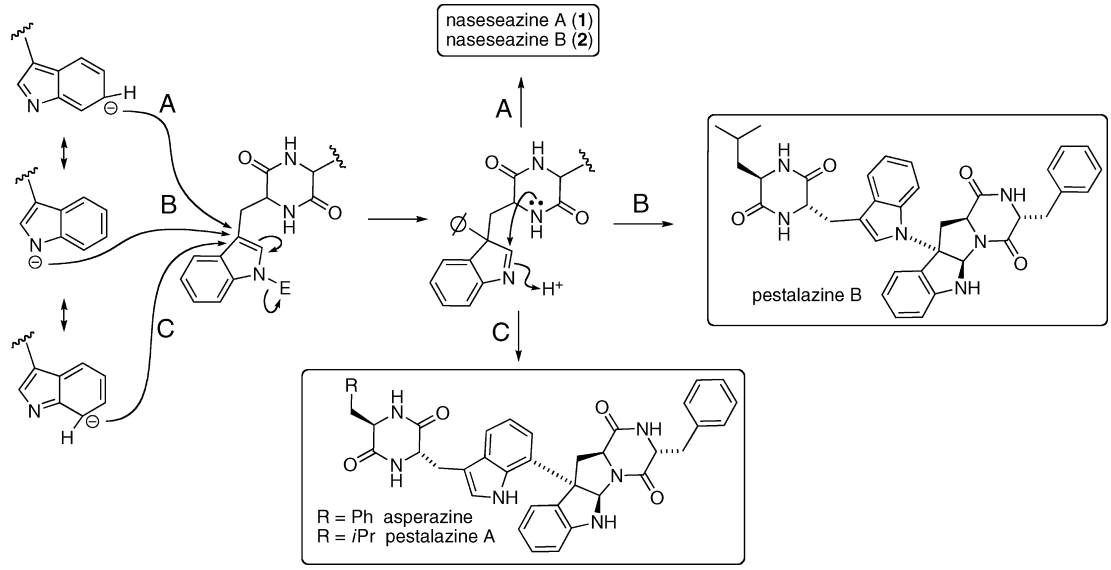


Figure 3. A plausible biosynthetic route to the naseseazines and related dimeric diketopiperazines.

be noncytotoxic in MTT anticancer assays against AGS (gastric adenocarcinoma), SH-SY5Y (neuroblastoma), TF-1 (erythroleukemia), and HT-29 (colorectal adenocarcinoma) cell lines. As the traditional role proposed for novel microbial secondary metabolites is one of chemical defense against competing microbes, the absence of obvious cytotoxic properties may, at first glance, be viewed as limiting the ecological and hence biological utility of the naseseazines. We take a broader view and note that noncytotoxic biological interactions are critical to many important ecological processes (e.g., pheromones, allemones) and pharmacologies (e.g., immunosuppressants, pain relief, hypertension) and suggest that the noncytotoxic naseseazines in all probability possess a more subtle, alternative, and as yet undefined biology.

The naseseazines represent an extension of the DKP pharmacophore into novel dimeric, noncytotoxic, and chemically stable space, providing synthetic and medicinal chemists with attractive new targets for developing and elaborating on the DKP scaffold as a means to support future drug discovery. Furthermore, the plausible biosynthetic route outlined in Figure 3 suggests a possible biomimetic entry into this structure class. Manipulation of the electronegativity of suitably substituted aromatic nucleophiles could drive regioselective C-3 substitution, with concomitant elimination of an N-1 electron-withdrawing substituent, and subsequent cyclization from N-10 to C-2, to generate dimeric DKPs bearing an aromatic C-3 carbon−carbon linkage and a pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indole-1,4-dione heterocycle.

Acknowledgment. We thank F. Lafi and L. Sly (UQ) for the taxonomic analysis and A. Jones (UQ) for assistance with mass spectrometry. R.R. acknowledges the provision of an Australian Postgraduate Award. The research was funded in part by the Institute for Molecular Bioscience and UQ. We thank the Fijian Government for permission to collect sediment samples.

Supporting Information Available: Full details of collection, extraction and isolation, ^{1}H NMR spectra and tabulated 1D and 2D NMR data for naseseazines, in methanol-d_4 and DMSO-d_6. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901466R