Bromopyrrole alkaloids from the Jamaican sponge *Didiscus oxeata*

Jin-Feng Hu[a], Jiangnan Peng[a], Abul B. Kazi[a], Michelle Kelly[b] and Mark T. Hamann[a]*

[a]*Department of Pharmacognosy and National Center for Natural Products Research (NCNPR), School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

[b]*National Center for Aquatic Biodiversity and Biosecurity, National Institute of Water & Atmospheric Research (NIWA) Ltd, Newmarket, Auckland, New Zealand*

Three bromopyrrole alkaloids (**1**–**3**) were isolated from the acetone extracts of *Didiscus oxeata* during chemical and biological investigation of Caribbean and Indo-Pacific marine sponges. The structures were established by spectroscopic methods. Mukanadin D (**3**) was obtained for the first time as a naturally-occurring C_{11} bromopyrrole alkaloid.

Keywords: bromopyrrole alkaloids, mukanadin D, *Didiscus oxeata*

In the course of our chemical and biological investigations of Caribbean and Indo-Pacific marine sponges, we have recently reported antiinfective cyclic peroxides,[1] manzamine-type alkaloids,[2-4] and sterols.[5] Brominated metabolites have also emerged from the marine environment as a large class of bioactive marine natural products. Examples of these include the bromo-alkaloids with cytotoxicity, antimalarial, and antitubercular activities.[6-10] We report here the isolation of the bromopyrrole alkaloids (**1**–**3**) from the acetone extracts of the Jamaican sponge *Didiscus oxeata* Hechtel (Order Halichondrida, Family Desmoxyiidae). The known C_5 bromopyrrole (**1**) was previously isolated in 1997 by Pietra, *et al*.[11] from the Axinellid sponge *Acanthella carteri* where it co-occurred with C_9 and C_{11} bromopyrrole-derived alkaloids. The known C_{11} mukanadin B (**2**) was isolated from the Okinawan marine sponge *Agelas nakamurai* in 1999 by Kobayashi, *et al*.[12] Compound **3** was previously synthesised as a mixture of C-10 double bond isomers by Lindel and Hoffmann in 1997.[13] In this paper, mukanadin D (**3**) was obtained for the first time as a naturally-occurring C_{11} bromopyrrole alkaloid. The trival name assigned to **3** is based on the previous work by Kobayashi.[12] Many bromopyrrole metabolites have been detected from marine sponges,[14-18] and some of them exhibited bioactivities such as antiplasmodial and cytotoxicity,[14] as well as anti-histamine activities.[15] However, our isolated bromopyrroles (**1**–**3**) were assayed for their antimalarial, antimicrobial, antitubercular, and anticancer activities, and found inactive.

Both compounds **1** and **2** were obtained as amorphous white solids, and the structures were identified by comparison of their MS, ^{1}H and ^{13}C NMR data with the literature.[11,12] Compound **3** showed an isotope pattern of 1:2:1 in the LRESI-FTMS (negative mode) spectrum for the pseudo-molecular ([M-H]$^-$) ion peaks at *m*/*z* 405, 407 and 409, indicating the presence of two bromine atoms in the molecule. The molecular formula of **3** was determined as $C_{11}H_{10}Br_2N_4O_3$ by HR-ESIMS at *m*/*z* 404.9038 [M-H]$^-$ and supported by its ^{1}H and ^{13}C NMR data. The ^{1}H NMR spectrum (DMSO-d_6) of **3** showed general features similar to those of **2**. Analysis of ^{1}H-^{1}H COSY NMR data showed the existence of a proton spin system: –CONHCH$_2$CH$_2$CH= (H-7, H$_2$-8, H$_2$-9, H-10) at δ 8.18 (1H, t, *J* = 5.6 Hz), 3.24 (2H, m), 2.36 (2H, dt, *J* = 7.5, 6.8 Hz), and 5.54 (1H, t, *J* = 7.5 Hz). One bond proton-carbon connectives were determined by an HMQC experiment. A heteronuclear multiple bond (^2J or ^3J) correlation (HMBC) NMR indicated long range proton-carbon couplings (Fig. 1). Exchangeable signals (D$_2$O) in the ^{1}H NMR spectrum led to unambiguous assignments of ^{1}H and ^{13}C NMR chemical shifts as well as the relative stereochemistry of **3** as in the recently reported africantriol isolated from *Streptomyces* sp.[19] The ^{1}H NMR spectrum recorded in DMSO-d_6 of **3** showed exchangeable signals of NH groups at δ11.75 (H-1), 8.18 (H-7), 10.15 (H-13), and 10.91 (H-15), which could be confirmed by the observed HMBC correlations (Fig. 1). This led to the chemical shifts assignments for all protons and carbons. The *cis*-configuration of the C-10 double bond was confirmed on the basis of the NOE between H-9 at δ2.36 and H-15 at δ10.91 (Fig. 1).

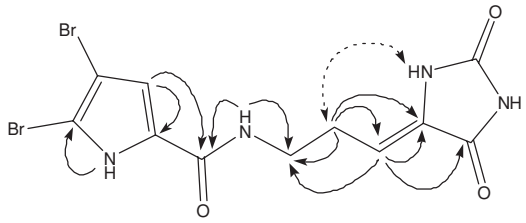


Fig. 1 Key HMBC (H→C) and NOE (H⇠⇢H) correlations of **3**.

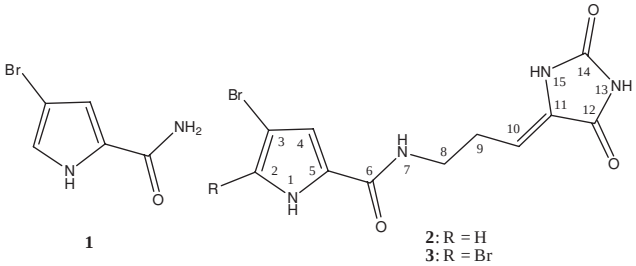
Experimental

General experimental procedures

1D and 2D NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals with resonances at δ_H/δ_C 2.49/39.5 (DMSO-d$_6$). ESI-FTMS analyses were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron HR HPLC-FT spectrometer by direct injection into an electrospray interface. Silica gel (200–400 mesh) and Sephadex LH-20 (Pharmacia) for column chromatography was obtained from Natland International Corporation (www.natland.com) and SIGMA Chemical Co. (USA), respectively. TLC was performed on aluminum sheets (silica gel 60 F_{254}, Merck KGaA, Germany).

Collection, identification and taxonomy of sponge Didiscus oxeata

The sponge was collected from caves and vertical coral walls at Rio Bueno, Jamaica, on 12 July 2000. The sponge forms a massive semispherical mass with a brain-like grooved surface. The texture is pulpy and the sponge easily crushed. The colour in life is orange; the interior is lighter in colour. The sponge produces copious amounts of mucus and has filamentous algal epibionts on the surface. The skeleton consists of fine widely spaced tracts of oxea in **2** size



* Correspondent. E-mail: mthamann@olemiss.edu

categories, with a palisade of smaller oxea on the surface. Curved "anisocaliculate" microrhabds are common throughout the sponge. The sponge is *Didiscus oxeata* Hechtel, 1983 (Order Halichondrida, Family Desmoxyiidae). A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 2001.7.20.1).

Extraction and isolation of bromopyrroles

The sponge was immediately frozen then freeze-dried (1.5 kg) prior to extraction with acetone (4 l) in a blender. After filtration and evaporation of the acetone, the combined extracts were dried to give a brownish gum (1.5 g). This material was chromatographed on Si gel (column: 4.5 × 62 cm) with a $CHCl_3$–MeOH gradient (20:1–1:1) to yield six fractions: compounds **1** (80 mg, 5×10^{-5}%), **2** (5 mg, 2×10^{-6}%) and **3** (5 mg, 2×10^{-6}%) were obtained from fr. 4 ($CHCl_3$–MeOH 8:1–3:1, *ca* 400 mg) and was further purified by gel permeation chromatography on Sephadex LH-20 (column: 4.9 × 76 cm) in MeOH.

Mukanadin D (**3**). Brown solid. ^{1}H NMR (DMSO-d_6): δ11.75 (1H, brs, H-1, NH), 6.92 (1H, s, H-4), 8.18 (1H, t, *J* = 5.6 Hz, H-7, NH), 3.24 (2H, m, H-8), 2.36 (2H, dt, *J* = 7.5, 6.8 Hz, H-9), 5.54 (1H, t, *J* = 7.5 Hz, H-10), 10.15 (1H, brs, H-13, NH), and 10.91 (1H, brs, H-15, NH). ^{13}C NMR (DMSO-d_6): δ110.0 (C-2, s), 95.7 (C-3, s), 112.2 (C-4, d), 127.7 (C-5, s), 160.6 (C-6, s), 38.5 (C-8, t), 27.7 (C-9, t), 109.8 (C-10, d), 132.1 (C-11, s), 165.1 (C-12, s), 155.7 (C-14, s). ESI-FTMS, *m*/*z* = 405, 407, 409 ([M - H]$^-$, 1:2:1). HRESI-FTMS, *m*/*z* = 404.9038, $C_{11}H_9{}^{79}Br^{81}BrN_4O_3$ [M - H]$^-$ requires *m*/*z* = 404.9021.

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References

1 J.-F. Hu, H.-F. Gao, M. Kelly and M.T. Hamann, *Tetrahedron*, 2001, **57**, 9379.
2 M. Yousaf, K.A. El Sayed, K.V. Rao, C.W. Lim, J.-F. Hu, M. Kelly, S.G. Franzblau, F. Zhang, O. Peraud, R.T. Hill and M.T. Hamann, *Tetrahedron*, 2002, **58**, 7397.
3 J. Peng, J.-F. Hu, A.B. Kazi, S.G. Franzblau, F. Zhang, R.F. Schinazi, S.S. Wirtz, P. Tharnish, M. Kelly and M.T. Hamann, *J. Am. Chem. Soc*., 2003, **125**, 13382.
4 J.-F. Hu, M.T. Hamann, R.T. Hill and M. Kelly, *The Alkaloids: Chemistry and Biology*, eds Geoffrey A. Cordell, Academic Press, San Diego, 2003. Vol. 60, pp. 207 - 285.
5 J.-F. Hu, M. Kelly and M.T. Hamann, *Steroids*, 2002, **67**, 743.
6 H.-F. Gao, M. Kelly, M.T. Hamann, *Tetrahedron*, 1999, **55**, 9717.
7 J.-F. Hu, J.A. Schetz, M. Kelly, J. Peng, K.K.H. Ang, H. Flotow, C.Y. Leong, S.B. Ng, A.D. Buss, S.P. Wilkins and M.T. Hamann, *J. Nat. Prod*., 2002, **65**, 476.
8 K.A. El Sayed, P. Bartyzel, X.Y. Shen, T.L. Perry, J.K. Zjawiony and M.T. Hamann, *Tetrahedron*, 2000, **56**, 949.
9 M.T. Hamann, *Curr. Pharm. Design*, 2003, **9**, 879.
10 D.J. Gochfeld, K.A. El Sayed, M. Yousaf, J.-F. Hu, P. Bartyzel, D.C. Dunbar, S.P. Wilkins, J.K. Zjawiony, R.F. Schinazi, S.S. Wirtz, P.M. Tharnish and M.T. Hamann, *Mini Reviews in Medicinal Chemistry*, 2003, **3**, 401.
11 I. Mancini, G. Guella, P. Amade, C. Roussakis and F. Pietra, *Tetrahedron Lett.*, 1997, **38**, 6271.
12 H. Uemoto, M. Tsuda and J. Kobayashi, *J. Nat. Prod.*, 1999, 62, 1581.
13 T. Lindel and H. Hoffmann, *Liebigs Ann./Recueil*, 1997, 1525.
14 G.M. Koenig, A.D. Wright and A. Linden, *Planta Med.*, 1998, **64**, 443.
15 F. Cafieri, E. Fattorusso, A. Mangoni and O. Taglialatela-Scafati, *Tetrahedron Lett.*, 1996, **37**, 3587.
16 T. Iwagawa, M. Kaneko, H. Okamura, M. Nakatani, R.W.M. Van Soest, *J. Nat. Prod.* 1998, **61**, 1310.
17 F.J. Schmitz, S.P. Gunasekera, V. Lakshmi, L.M.V. Tillekeratne, *J. Nat. Prod.*, 1985, **48**, 47.
18 H. Tada and T. Tozyo, *Chem. Lett.*, 1988, 803.
19 J.-F. Hu, D. Wunderlich, R. Thiericke, H.-M. Dahse, S. Grabley, X.-Z. Feng and I. Sattler, *J. Antibiot*., 2003, **56**, 747.