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20-*N*-Methylpurpuramine E: New Bromotyrosine-Derived Metabolite from Okinawan Marine Sponge *Pseudoceratina purpurea*

Toshiaki Teruya, Arihiro Iwasaki, and Kiyotake Suenaga*

Department of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522

Received March 18, 2008; E-mail: suenaga@chem.keio.ac.jp

A new bromotyrosine-derived metabolite, 20-*N*-methylpurpuramine E (**1**), was isolated from the marine sponge *Pseudoceratina purpurea*. The structure was elucidated by spectroscopic analysis. 20-*N*-Methylpurpuramine E (**1**) showed weak cytotoxicity against HeLa S_3 cells.

Marine sponges of the Verongida are a rich source of bromotyrosine-derived compounds[1,2] that show various biological activities such as antifouling,[3,4] cytotoxicity,[5–7] antibacterial,[5] and Na, K-ATPase inhibition.[4] In our continuing search for new substances from marine sponges, we investigated the constituents of marine sponge *Pseudoceratina purpurea* collected in Okinawa Prefecture and isolated a new bromotyrosine-derived metabolite, 20-*N*-methylpurpuramine E (**1**). In this report, we describe the isolation, structural elucidation, and biological activity of 20-*N*-methylpurpuramine E (**1**) (Chart 1).

The Okinawan sponge *Pseudoceratina purpurea* (480 g, wet weight) was extracted with methanol (1 L) for 7 days. The extract was filtered, concentrated, and partitioned between EtOAc and H_2O. The EtOAc-soluble material was further partitioned between aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to fractionation using ODS silica gel (40% aqueous MeOH to MeOH) and reverse-phase HPLC (Develosil ODS-HG-5, 60% aqueous MeOH, 0.1% TFA) to give 20-*N*-methylpurpuramine E (**1**) as a yellow oil along with purpuramine E (**2**).[9] 20-*N*-Methylpurpuramine E (**1**) exhibited cytotoxicity against HeLa S_3 cells, with an IC_{50} value of 4.3 μg mL^{-1}.

20-*N*-Methylpurpuramine E (**1**) exhibited a 1:2:1 ion cluster peak at *m*/*z* 540/542/544, indicating the presence of two bromine atoms. The molecular formula of **1** was found to be $C_{22}H_{27}Br_2N_3O_3$ by HRESIMS (*m*/*z* 540.0485 calcd for $C_{22}H_{28}{}^{79}Br_2N_3O_3$ $[M+H]^+$ 540.0497). The NMR data for **1** are summarized in Table 1. The ^{1}H NMR spectrum exhibited signals assignable to a mono-substituted benzene ring (δ_H 7.21, 7.20, and 7.13), a 3-aminopropanol (δ_H 4.07, 3.45, and 2.26), a 2-aminoethyl (δ_H 3.41 and 2.72), an isolated methylene (δ_H 3.88), and a symmetrically substituted aromatic ring (δ_H 7.43). The ^{13}C NMR spectrum and the HMQC spectrum of **1** showed that there were fourteen carbons consisting of seven sp^2 methine carbons (δ_C 134.4, 134.4, 129.9, 129.9, 129.3, 129.3, and 127.2), and seven sp^2 carbons (δ_C 166.0, 153.2, 152.1, 140.3, 138.1, 118.7, and 118.7), six saturated methylene carbons (δ_C 71.0, 57.0, 41.3, 35.2, 29.9, and 26.3), and two methyl carbons (δ_C 43.6 and 43.6). Amide and oxime functionalities were indicated by IR absorption at 3400, 3029, 1671, and 1495 cm^{-1}. The carbon signals at δ_C 166.0 (C8) and 153.2 (C9) could be assigned to the amide–oxime conjugated system, which was supported by the presence of two exchangeable proton signals at δ_H 11.67 and 7.90 in the ^{1}H NMR spectrum measured in DMSO-d_6. A detailed analysis of the COSY spectrum of **1** allowed three partial structures, C1–C5, C10–C11, and C18–C20, to be constructed, as shown in Figure 1. The connectivity of three partial structures was clarified by HMBC correlations: H1/C7, H7/C6, H7/C8, H7/C9, H10/C9, H11/C12, H11/C13, H13/C14, H13/C15, H13/C17, H18/C15, H20/C21, and H21/C20. The upfield ^{13}C NMR chemical shift of C-7

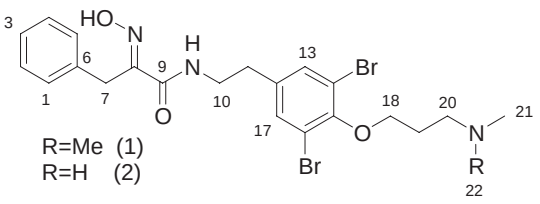

Chart 1.

Table 1. NMR Data for **1** in CD_3OD

No.	^{1}H (ppm)[a),b)]	^{13}C (ppm)[c)]
1, 5	7.21 m	129.9
2, 4	7.20 m	129.3
3	7.13 m	127.2
6		138.1
7	3.88 s	29.9
8		153.2
9		166.0
10	3.41 t (6.8)	41.3
11	2.72 t (6.8)	35.2
12		140.3
13, 17	7.43 s	134.4
14, 16		118.7
15		152.1
18	3.45 t (7.2)	71.0
19	2.26 m	26.3
20	4.07 t (5.6)	57.0
21, 22	2.94 s	43.6

a) Recorded at 400 MHz. b) Coupling constants (Hz) are in parentheses. c) Recorded at 100 MHz.

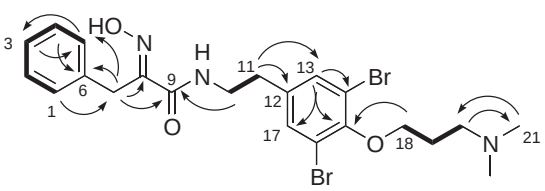

Figure 1. Partial structure of **1**, based on 2D NMR correlations.

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in 20-*N*-methylpurpuramine E (δ_C 29.9) suggests an E configuration for the oxime[8] (the corresponding chemical shift in the case of (*Z*)-oxime is >35 ppm).[9] Thus, the gross structure of **1** was determined as shown in Figure 1.

In conclusion, 20-*N*-methylpurpuramine E (**1**), a novel bromotyrosine-derived compound was isolated from the Okinawan sponge *Pseudoceratina purpurea*. The structure of 20-*N*-methylpurpuramine E (**1**) is closely related to purpuramine E.[10] The C1–C9 unit present in the bromotyrosine-derived metabolites obtained thus far have been exclusively derived from bromotyrosine, except for purpuramines A–E.[10] The oxime function of purpuramines A–E and 20-*N*-methylpurpuramine E (**1**) is derived from phenylalanine. 20-*N*-Methylpurpuramine E (**1**) showed weak cytotoxicity against HeLa S_3 cells at $IC_{50} = 4.3\,\mu g\,mL^{-1}$.

Experimental

General Procedures. NMR spectra were recorded on a JNM-A400 [400 MHz (^{1}H) and 100 MHz (^{13}C)] spectrometer. The ^{1}H and ^{13}C chemical shifts are referenced to the solvent peaks (δ_H 3.31 and δ_C 49.5 in methanol-d_4, δ_H 2.49 in DMSO-d_6). High-resolution mass spectra (HRMS) were obtained on a PE Sciex QSTAR mass spectrometer. Column chromatography was performed on a ODS gel (Nacalai Tesque, Cosmosil 75C_{18}-OPN). Reverse-phase high-performance liquid chromatography (HPLC) was carried out on a Develosil ODS-HG-5 column (Nomura Chemical Co., Ltd.).

Isolation and Purification. The frozen sponge of *Pseudoceratina purpurea* (480 g), which was collected at −1 m off Bise Okinawa in November, 2006, was extracted with methanol (1.5 L) for seven days. The extracts were concentrated and partitioned between ethyl acetate and water. The materials obtained from the organic layer were partitioned between 90% methanol/H_2O and hexane. The aqueous methanol fraction (590 mg) was first separated by column chromatography on ODS (6 g) using 40% methanol, 60% methanol, 80% methanol, and methanol. The fraction (140 mg) eluted with 60% methanol was subjected to HPLC [Deverosil ODS-HG-5 (250 × 20 mm); flow rate 5 mL min^{-1}; detection, UV 215 nm; solvent 60% methanol/0.1% trifluoroacetic acid] to give 20-*N*-methylpurpuramine E (**1**) (13.1 mg, retention time 25 min).

20-*N*-Methylpurpuramine E (1). Yellow oil; IR (thin film) ν_{max} 3400, 3029, 1671, and 1495 cm^{-1}; ^{1}H NMR and ^{13}C NMR in CD_3OD, see Table 1. ^{1}H NMR in DMSO-d_6; δ 11.67 (1H, s, 8-NOH), 7.90 (1H, t, $J = 6.0$ Hz, 9-NH), 7.48 (2H, s, H-13, H-17), 7.23 (2H, m, H-1, H-5), 7.15 (3H, m, H-2, H-3, H-4), 3.99 (2H, t, $J = 5.2$, H-20), 3.80 (2H, s, H-7), 3.37 (2H, m, H-18), 3.33 (2H, m, H-10), 2.83 (6H, s, H-21, H-22), 2.74 (2H, t, $J = 6.8$, H-11), 2.16 (2H, m, H-19); HRMS (ESI) Exact mass calcd for $C_{22}H_{28}{}^{79}Br_2N_3O_3$ $[M + H]^+$ requires m/z 540.0497. Found m/z 540.0485.

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