# New Moloka'iamine Derivatives from an Undescribed Verongid Sponge

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The polar extract of an undescribed Verongid sponge from the island of Molokai yielded three new bromotyramines. Two compounds terminate in a chlorocyclopentanedione enamine moiety. Their structures were elucidated by NMR spectra measured at −30 °C. The third compound is the *N*-methyl derivative of a known compound. This structure was determined by NMR spectra measured at room temperature.

In 1993 we reported[1] isolation of a bromotyramine, moloka'iamine (**1**), from an undescribed genus of a Verongid sponge, which we had collected off the pier pilings in Kaunakakai Harbor on the island of Molokai. It was the major constituent of the sponge and the only compound whose structure is compatible with those normally found in Verongid sponges. The minor compounds were of mixed terpenoid−shikimate biogenesis. An intriguing property of this sponge, which for lack of a binomial we call "Moloka'i sponge", is its release of hydrogen cyanide when the sponge is broken apart after removal from the ocean. None of the characterized metabolites offered a clue to this phenomenon. A recent report by Fusetani and co-workers[2] of the isolation of moloka'iamine (**1**) and of ceratinamine (**2**), which is a cyanoformamide derivative of **1**, from a sponge *Pseudoceratina purpurea*, prompted us to reexamine the Moloka'i sponge. Interestingly, the sponge zoologist who had examined the sponge described the animal as most comparable to the genera *Psammaplysilla* and *Pseudoceratina*.[1] Both genera are in the family Aplysinellidae, order Verongida. Ceratinamine (**2**) might well be a biogenetic precursor for the observed emission of hydrogen cyanide. This idea was recently articulated by Schoenfeld and Ganem in connection with their synthesis of **2**.[4] Reexamination of a sample of Moloka'i sponge collected on the Wai'anae coast of Oahu furnished, in addition to moloka'iamine (**1**), *N*-methylceratinamine (**3**) and two new molokaiamine derivatives, wai'anaeamines A (**4**) and B (**5**), and the known psammaplysin A (**6**).[3] Interestingly, the distinctive nitrogen substituent in **4** and **5** is the HCl adduct of a cyclopentanedione end group, which we had previously encountered in psammaplysin E (**7**), a constituent of an *Aplysinella* sp. sponge from Pingelap atoll, Micronesia.[5] Fu and Schmitz[6] have recently reported the isolation of 7-hydroxyceratinamine from a Micronesian Aplysinella, from which Schmitz and co-workers had earlier isolated psammaplysins.[7] With the isolation of psammaplysin E and the wai'anaeamines, it has become evident that the chemical makeup of Moloka'i sponge mirrors its taxonomic relationship to the two aplysinellid genera *Psammaplysilla* and *Pseudoceratina*. The same five metabolites were also isolated from a recent recollection of Moloka'i sponge in Kaunakakai Harbor.

The freeze-dried sponge was blended with methanol and the extract partitioned between methanol and hexanes. The methanol phase was concentrated and the residue was subjected first to reversed-phase vacuum liquid chromatography ) (VLC), followed by reversed-phase HPLC. Elution with acetonitrile−water−TFA (2:8:0.005) yielded wai'anaeamines A (**4**) and B (**5**) and *N*-methylceratinamine (**3**). HRFABMS data for the wai'anaeamines provided identical molecular formulas of $C_{17}H_{19}Br_2ClN_2O_3$. NMR data readily identified the tyrosine portion of the molecules. However, no signals were observed for C-13, C-14, C-17, or H-12, which did not allow structural identification of the nitrogen substituent. Furthermore, H-15 and H-16 signals were too broad for meaningful COSY, HMQC, or HMBC experiments. The signals for the chlorocyclopentanedione moiety that did appear at room temperature were duplicated. The duplication and broadening of the signals is most likely caused by *E*/*Z* isomerization at C-12. Raising the temperature to 75 °C converged some of the duplicated signals, but signal broadening was still too prominent to see correlations in the 2D spectra. However, lowering the temperature to −30 °C produced the missing and sharpened the broadened signals. NMR data for **4** and **5** are summarized in Tables 1 and 2.

$^{13}$C Assignments for C-14 and C-17 were deduced from HMBC two-bond correlations. In Table 1, one of the isomers

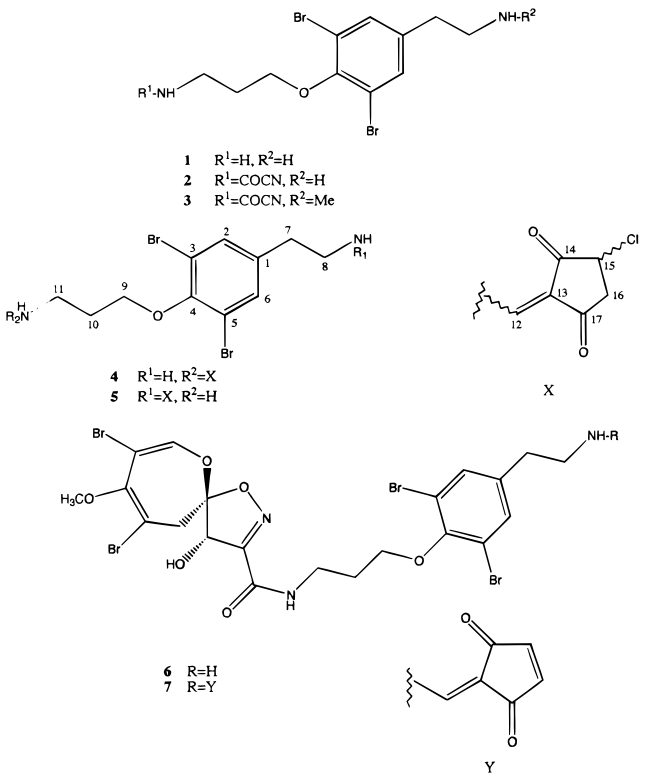


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**Table 1.** NMR Data for Wai'anaeamine A (**4**) in $CD_3OD$ at −30 °C

| no. | $^{13}C$ (ppm) | $^{1}H$ (ppm) | COSY correlations | HMBC correlations[b] |
|---|---|---|---|---|
| 1 | 138.4 | | | |
| 2, 6 | 134.9 | 7.48, 7.49 (2H, 2s) | | 152.7 |
| 3, 5 | 119.2 | 7.48 | | *7.48* |
| 4 | 152.7 | 4.08, 7.48 | | |
| 7 | 35.9 | 2.92 (2H, m) | 3.70 | 134.9, 138.4, *7.48* |
| 8 | 52.9 | 3.70 (2H, m) | 2.92 | 138.4, 158, *7.57*, *7.65* |
| 9 | 71.5 | 4.08 (2H, t, 5.7 Hz) | 2.17 | 29.0, 38.7 |
| 10 | 29.0 | 2.17 (2H, m) | 4.08, 3.27 | 71.5, 38.7, *4.08*, 3.27 |
| 11 | 38.7 | 3.27 (2H, t, 7.4 Hz) | 2.17 | |
| 12 | 158.1 | 7.57 (0.5H, s) | | 201.6, 197.9, 52.9, *3.70* |
| | 157.9 | 7.65 (0.5H, s) | | 200.0, 199.2, 52.9 |
| 13 | 106.3 | | | *7.65* |
| | 106.1 | | | *7.57* |
| 14[a] | 199.2 | | | *7.65* |
| | 197.9 | | | 3.14, 4.6 |
| 15 | 55.8 | 4.57 (0.5H, dd, 8.4, 3.6 Hz) | 3.11, 3.14 | 197.9, *7.65* |
| | 54.9 | 4.61 (0.5H, dd, 8.4, 3.6 Hz) | | |
| 16 | 45.7 | 3.11 (0.5H, dd, 18.0, 8.3 Hz) | 2.56, 4.57 | |
| | | 2.46 (0.5H, dd, 18.0, 3.6 Hz) | 3.11 | |
| | 45.1 | 3.14 (0.5H, dd, 18.0, 8.3 Hz) | 2.60, 4.57 | |
| | | 2.60 (0.5H, dd, 18.0, 3.6 Hz) | 3.14 | |
| 17[a] | 201.6 | | | *7.57*, *3.14*, *2.6* |
| | 200.0 | | | *3.11* |

[a] Data for C-14 and C-17 may be interchanged. [b] HMBC correlations shown in part structures **A** and **B** supported pentanedione structure and its relative position in **4** and **5**.

**Table 2.** NMR Data for Wai'anaeamine B (**5**) in $CD_3OD$ at −30 °C

| no. | $^{13}C$ (ppm) | $^{1}H$ (ppm) | COSY correlations | HMBC correlations[b] |
|---|---|---|---|---|
| 1 | 137.8 | | | |
| 2, 6 | 134.5 | 7.56, 7.57 (2H, 2s) | | 119.5, 137.8, 153.1 |
| 3, 5 | 119.5 | | | |
| 4 | 153.1 | | | |
| 7 | 33.2 | 2.89 (2H, t, 7.3 Hz) | 3.14 | 41.4, 134.9, 138.4 |
| 8 | 41.4 | 3.14 (2H, m) | 2.89 | 33.1 |
| 9 | 71.0 | 4.05 (2H, m) | 2.22 | 49.8, 31.0 |
| 10 | 31.0 | 2.22 (2H, m) | 4.05, 3.90 | 49.8 |
| 11 | 49.8 | 3.90 (2H, m) | 2.22 | |
| 12 | 158.6 | 8.13 (0.5H, s) | | 106, *3.90* |
| | 158.4 | 8.16 (0.5H, s) | | |
| 13 | 106.5 | | | |
| | 106.1 | | | |
| 14 | 199.4 | | | *8.16* |
| | 198.0 | | | *3.14*, *4.60*, *8.13* |
| 15 | 55.8 | 4.60 (0.5H, dd, 8.6, 3.7Hz) | 3.14, 2.58 | 197.9, *7.65* |
| | 55.0 | 4.62 (0.5H, dd, 9.2, 3.7 Hz) | | |
| 16 | 45.6 | 3.14 (1H, m) | 3.14, 2.58 | 55 |
| | 45.1 | 2.58 (1H, 2dd, 18.9, 3.8 Hz) | | |
| 17[a] | 201.9 | | | *8.13*, *3.14* |
| | 200.0 | | | *8.16*, *2.58* |

[a] Data for C-14 and C-17 may be interchanged. [b] HMBC correlations shown in part structures **A** and **B** supported pentanedione structure and its relative position in **4** and **5**.

of **4** has a $^{13}C$ shift of 201.6 ppm, which gives strong correlations to $^{1}H$ signals at 3.14 and 2.60 ppm belonging to H-16. Also $^{1}H$ signals at 3.14 and 4.61 ppm correlate with a $^{13}C$ signal at 197.9 ppm. By deduction, we may assign these $^{13}C$ shifts for C-17 and C-14, respectively. In addition, the H-12 shift of 7.57 ppm belongs to this isomer. In the other isomer, the $^{13}C$ signal at 200.0 ppm correlates with the $^{1}H$ signal at 3.11 ppm of H-16. If this signal is assigned for C-17, the remaining signal of 199.2 ppm is assigned to C-14.

In Table 2, similar deductions may be made to assign signals to C-14, C-17, and H-12 of the respective isomers without specifying the geometries. Part structures **A** and **B** show HMBC correlations for **4** and **5**, respectively.

HRFABMS data for *N*-methylceratinamine provided a molecular formula of $C_{14}H_{17}Br_2N_3O_2$. NMR data showed close correlation to ceratinamine, the structural similarity was confirmed by COSY, HMQC, and HMBC experiments. The signals for the methylene (C-8) vicinal to the *N*-methyl are shifted relative to ceratinamine. The presence of the *N*-methyl and its correlation to C-8 are also shown by the 2D NMR experiments. NMR data for **3** are summarized in Table 3.

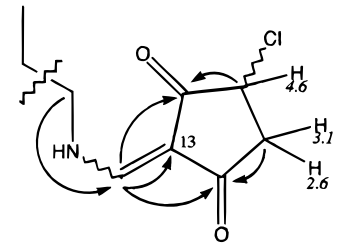
## Experimental Section

**General Experimental Procedures.** All NMR spectra were recorded on a General Electric GN Omega 500 MHz NMR spectrometer. The IR spectra were measured on a Perkin−Elmer 1420 spectrometer in $CHCl_3$. UV spectra were measured with a Hewlett−Packard 8452A diode array spectrophotometer. FABMS were measured on a JEOL JMX-SX102/SX102 tandem mass spectrometer using glycerol as matrix. Optical rotations were measured on a JASCO DIP-370 digital polarimeter.

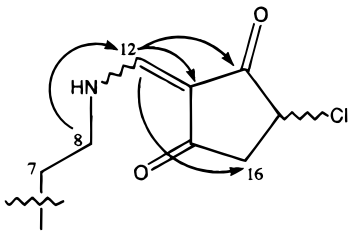
**Animal Material.** Pending taxonomic classification of the sponge, voucher samples are retained at the University of Hawaii at Manoa, Department of Chemistry. For description notes, see Hamann and Scheuer.[1]

**Table 3.** NMR Data for *N*-Methylceratinamine (**3**) in DMSO at Room Temperature

| no. | $^{13}$C (ppm) | $^{1}$H (ppm) | COSY correlations | HMBC correlations |
|---|---|---|---|---|
| 1 | 136.6 | | | 2.85 |
| 2,6 | 133.3 | 7.59 (2H, s) | | *2.85*, *7.59*, 151, 117 |
| | | | | 30 |
| 3,5 | 117.6 | | | 7.59 |
| 4 | 151.2 | | | 3.94, 7.59 |
| 7 | 30.0 | 2.85 (2H, t, 7.5 Hz) | 3.14 | 7.59 |
| 8 | 48.6 | 3.14 (2H, m) | 2.89, 8.47 | 2.89 |
| 9 | 70.6 | 3.94 (2H, t, 6.0 Hz) | 1.98 | 1.98, 3.43 |
| 10 | 28.7 | 1.98 (2H, p, 6.5 Hz) | 3.94, 3.43 | 3.43, 3.94 |
| 11 | 36.8 | 3.43 (2H, t, 6.5 Hz) | 1.98 | 1.98, 3.94 |
| CO | 143.1 | | | 3.43 |
| CN | 112.4 | | | |
| (CO)NH | | 9.95 (1H, t, 5.0 Hz) | | |
| ($CH_3$)NH | | 8.47 (1H, br s) | 3.15, 2.55 | |
| $NCH_3$ | 32.6 | 2.55 (3H, s) | 8.47 | |




**A**




**B**

**Isolation of *N*-Methylceratinamine (3) and Wai'anaeamines A (4) and B (5).** Sponge samples (540 g, dry) were blended with MeOH and the extract partitioned between methanol and hexanes. The residue (19.25 g) from the methanol layer was partitioned between 1-butanol and water. The butanol layer was concentrated to a residue (908 mg), which was subjected to reversed-phase flash chromatography. The fraction (471 mg), which was eluted with acetonitrile−water (1:1), was further separated by reversed-phase HPLC using a Cosmosil 5C18-AR column and acetonitrile−water−TFA (2:8:0.005) as eluent. Compounds **4** (12.5 mg), **5** (10.8 mg), and **3** (10.1 mg) were isolated consecutively as white solids.

***N*-Methylceratinamine (3):** UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 278 (3.76), 285 (3.77) nm; IR (dry film) $\nu_{max}$ 1676, 1458, 1204 cm$^{-1}$; NMR data, Table 3; HRFABMS *m*/*z* 419.9731 (M + H)$^+$ (calcd for $C_{14}H_{18}Br_2N_3O_2$, 419.9745).

**Wai'anaeamine A (4):** $[\alpha]^{27}_D$ 2.6° (*c* 0.23, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 209 (4.36), 310 (3.89) nm; IR (dry film) $\nu_{max}$ 2921, 1685, 1618 cm$^{-1}$; NMR data, Table 1; HRFABMS *m*/*z* 494.9482 (M + H)$^+$ (calcd for $C_{17}H_{20}Br_2ClN_2O_3$, 494.9505).

**Wai'anaeamine B (5):** $[\alpha]^{27}_D$ −20.9° (*c* 0.067, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 209 (4.31), 309 (3.84) nm; IR (dry film) $\nu_{max}$ 1678, 1633, 1203 cm$^{-1}$; NMR data are shown in Table 2; HRFABMS: *m*/*z* 494.9572 (M + H)$^+$ (calcd for $C_{17}H_{20}Br_2ClN_2O_3$, 494.9505).

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**Supporting Information Available:** Copies of $^{1}$H, and $^{13}$C NMR, HMBC, MS, IR, and UV spectra and optical rotations of compounds **3**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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