7-Hydroxyceratinamine, a New Cyanoformamide-Containing Metabolite from a Sponge, *Aplysinella* sp.

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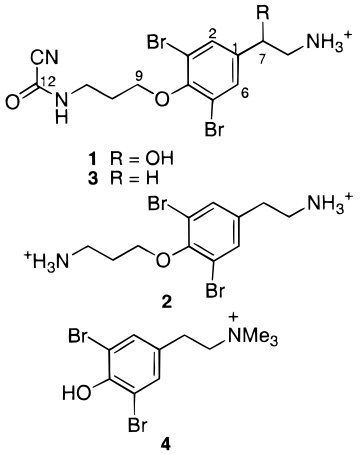
7-Hydroxyceratinamine (**1**) and the known compounds moloka'iamine (**2**), ceratinamine (**3**), and *N,N,N*-trimethyl-3,5-dibromotyramine (**4**), were isolated from a Micronesian sponge, *Aplysinella* sp. The structure of **1**, which contains the unusual cyanoformamide moiety, was solved by spectroscopic analysis.

In an earlier paper[1] we reported five bromotyrosine derivatives, psammaplysins A−C and E−F, from an *Aplysinella* sponge collected from Chuuk, Federated States of Micronesia. Our attention was later drawn to a more polar, but relatively small fraction remaining from our previous work, because this fraction showed marginal activity in an assay for inducement of apoptosis. Reversed-phase HPLC resolution of this polar fraction yielded a new compound, 7-hydroxyceratinamine (**1**), along with two known compounds, moloka'iamine (**2**)[2] and ceratinamine (**3**).[3] Additional moloka'iamine (**2**) and also *N,N,N*-trimethyl-3,5-dibromotyramine (**4**)[4] were isolated from the *n*-BuOH-soluble portion of the crude extract of this sponge.[1] Moloka'iamine (**2**) has been reported to possess a wide spectrum of bioactivity, such as antiviral,[2] antifouling,[3] and cytotoxic[2,3] activity; ceratinamine (**3**) exhibits both antifouling and cytotoxic activity;[3] and compound **4** displays dual adrenergic activity.[4] We report here the structure elucidation of 7-hydroxyceratinamine (**1**).



Compounds **2**−**4** were identified by comparison of their FABMS and NMR data with literature values.[2−4] ^{13}C NMR data for **4**, which were not reported earlier,[4] are herein reported.

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ +3.0° (*c* 0.8, MeOH). FABMS showed a cluster of peaks at *m/z* 420/422/424 (1:2:1), characteristic of a dibrominated compound. The molecular formula, $C_{13}H_{16}Br_2N_3O_3$, established by HRFABMS, had one oxygen more than that of ceratinamine (**3**). The ^{1}H NMR data (Experimental Section) for **1** were essentially identical to those of **3**, except that the two methylene triplets at δ 2.90 and 3.16 observed for ceratinamine (**3**) were missing in the ^{1}H NMR spectrum of **1**. Instead, a one-proton multiplet at δ 4.86 (H-7) and two one-proton doubled doublets ascribable to H-8 were observed. Consistent with this, the ^{13}C NMR spectrum of **1** showed signals at δ 69.2 (d, C-7), and 46.9 (t, C-8). Hence, compound **1** is a 7-hydroxylated product of **3**, namely 7-hydroxyceratinamine, and this was confirmed by HMBC correlations between H-7 and C-1 and C-2/6. The location of the cyanoformyl group was also substantiated by HMBC correlation between H-11 (δ 3.56) and C-12 (δ 145.2). No characteristic absorption for a cyano group was noted in the IR spectrum of **1**, whereas bands attributable to OH, NH, and amide were present. Lack of IR absorption for a cyano group was noted previously for **3**.[3]

7-Hydroxyceratinamine (**1**) represents the second example of naturally occurring cyanoformamide metabolites.[3] None of the pure compounds reported here showed significant activity in an apoptosis assay.[5]

Experimental Section

General Experimental Procedures. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. NMR spectra were recorded on a Varian VXR-500 spectrometer. MS were measured on a VG ZAB-E instrument. The IR spectrum was recorded on a Bio-Rad 3240-spc FT spectrophotometer; and the optical rotation was measured on a Rudolph Autopol III automatic polarimeter.

Animal Material. The animal material was described previously by Liu et al.[1]

Extraction and Isolation. Extraction of the 1995 collection of *Aplysinella* sp. (18T95) and preliminary chromatography on a Si gel column have been described in an earlier paper.[1] A minor fraction from the Si gel chromatography exhibited apoptosis activity, and was therefore rechromatographed by reversed-phase HPLC using 60% H_2O−MeOH containing 0.1% TFA as eluent to yield three amines: 7-hydroxyceratinamine (**1**) (9.5 mg), moloka'iamine (**2**) (5.1 mg), and ceratinamine (**3**) (3.6 mg). More moloka'iamine (**2**) was also obtained along with compound **4** (200 mg) from the *n*-BuOH-soluble fraction by chromatography on Si gel using $CHCl_3$−*n*-BuOH−HOAc−H_2O (1.5:6:1:1) as eluent.

7-Hydroxyceratinamine (1): amorphous solid; $[\alpha]_D$ +3.0° (*c* 0.8, MeOH); IR (neat) ν_{max} 3365, 3280, 1675, 1453, 1206, 1138 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.66 (2H, s, H-2 and H-6), 4.86 (1H, m, H-7), 4.04 (2H, t, J = 6 Hz, H-9), 3.56 (2H, t, J = 7 Hz, H-11), 3.16 (1H, dd, J = 3.5, 13 Hz, H-8), 2.95 (1H, dd, J = 9.5, 13 Hz, H-8′), 2.09 (2H, m, H-10); ^{13}C NMR (125 MHz, CD_3OD) δ 154.0 (s, C-4), 145.2 (s, C-12), 141.8 (s, C-1), 131.6 (d, C-2 and C-6), 119.4 (s, C-3 and C-5), 113.1 (s, C-13), 71.6 (t, C-9), 69.2 (d, C-7), 46.9 (t, C-8), 38.4 (t, C-11), 30.1 (t, C-10); FABMS *m/z* 420/422/424 (int, 1:2:1); HRFABS *m/z* 419.9568 (calcd for $C_{13}H_{16}N_3O_3{}^{79}Br_2$ 419.9558).

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Moloka'iamine (2): amorphous solid; FABMS *m*/*z* 351/353/355 (int, 1:2:1); ^{1}H and ^{13}C NMR data are in good agreement with literature values.[2]

Ceratinamine (3): amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.54 (2H, s, H-2 and H-6), 4.04 (2H, t, J = 6 Hz, H-9), 3.56 (2H, t, J = 6.5 Hz, H-11), 3.16 (2H, t, J = 7.5 Hz, H-8), 2.90 (2H, t, J = 7.5 Hz, H-7), 2.09 (2H, m, H-10); ^{13}C NMR (125 MHz, CD_3OD) δ 153.5 (s, C-4), 145.2 (s, C-12), 137.3 (s, C-1), 134.4 (d, C-2 and C-6), 119.4 (s, C-3 and C-5), 113.0 (s, C-13), 71.5 (t, C-9), 41.4 (t, C-8), 38.4 (t, C-11), 33.1 (t, C-7), 30.1 (t, C-10); FABMS *m*/*z* 404/406/408 (int, 1:2:1).

***N,N,N*-Trimethyl-3,5-dibromotyramine (4):** white needles (MeOH); mp 230 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.54 (2H, s, H-2 and H-6), 3.51 (2H, m, H-8), 3.11 (9H, s, N-Me), 2.95 (2H, m, H-7); ^{13}C NMR (125 MHz, DMSO-d_6) δ 149.9 (s, C-4), 132.8 (d, C-2 and C-6), 130.4 (s, C-1), 112.2 (s, C-3 and C-5), 65.4 (t, C-8), 52.3 (q, N-Me), 26.8 (t, C-7); FABMS *m*/*z* 336/338/340 (int, 1:2:1).

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