Isolation of 2-(3′-Bromo-4′-hydroxyphenol)ethanamine from the New Zealand Ascidian *Cnemidocarpa bicornuta*

Brent S. Lindsay,[†] Chris N. Battershill,[‡] and Brent R. Copp*[,†]

Department of Chemistry, University of Auckland, Private Bag 92019, Auckland, New Zealand, and National Institute of Water and Atmospheric Research, P.O. Box 14-901, Kilbirnie, Wellington, New Zealand

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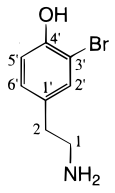
From the ascidian *Cnemidocarpa bicornuta*, 2-(3′-bromo-4′-hydroxyphenol)ethanamine (3′-bromotyramine) (**1**) has been isolated along with the previously reported sponge metabolite, 1,3-dimethylisoguanine. The structure of **1** was confirmed by synthesis.

As part of a recently initiated program to assess systematically the chemical diversity of New Zealand ascidians, we have investigated the chemistry of the common subtidal tunicate *Cnemidocarpa bicornuta* Sluiter, 1900 (Styelidae).[1] Although the methanolic extract of this organism was inactive in our initial bioassay screening, photodiode-array detected analytical reversed-phase HPLC indicated the presence of two major UV-absorbing components. Combinations of chromatography using reversed-phase C18 and Sephadex LH-20 solid supports afforded samples of these components, which were identified as the new natural product 2-(3′-bromo-4′-hydroxyphenol)ethanamine (**1**, 3′-bromotyramine) and the recently reported sponge metabolite 1,3-dimethylisoguanine (**2**).[2,3] Although halogenated β-phenethylamines are known from ascidian sources, for example, iodinated tyrosine derivatives,[4−6] the monobrominated tyramine moiety is often contained in more complex structures, most commonly in sponges (e.g., bastadins,[7,8] purpuramines,[9] and hemibastadinols[10]) but also in ascidians.[5,11] To the best of our knowledge, this is the first report of **1** as a natural product, though the compound has previously been synthesized.[12,13]



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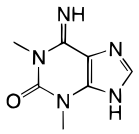

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A molecular formula for **1** of $C_8H_{10}NOBr$ was derived from HREIMS and from ^{1}H and ^{13}C NMR data. The ^{1}H NMR spectrum suggested the presence of a 1,2,4-trisubstituted benzene ring {δ 7.39 (d, J = 2.1 Hz), 7.07 (dd, J = 8.3, 2.1 Hz), and 6.87 (d, J = 8.3 Hz)} and a 1,2-disubstituted ethyl fragment {δ 3.11 and 2.84 (each 2H, t, J = 7.7 Hz)}. These fragments were confirmed by the observation in the ^{13}C NMR spectrum of three aromatic methine carbons (δ 134.34, 130.03, 117.65), three aromatic quaternary carbons (δ 154.69, 130.29, 111.13), and two alkyl CH_2 resonances (δ 41.99, 33.33). HMBC NMR data fully supported structure **1**, with crucial correlations being observed between ^{1}H resonances at δ 7.39 (H-2′) and δ 7.07 (H-6′) and the deshielded aromatic quaternary carbon C-4′ (δ 154.69) and the alkyl resonance C-2 at δ 33.33. Further confirmation of the structure was achieved by synthesis using a literature preparation,[12] which yielded a product that was identical with the natural product in all respects (^{1}H NMR, ^{13}C NMR, MS, HPLC co-injection, HPLC UV spectrum).

Compound **1** exhibited mild cytotoxicity to the P-388 murine leukemia cell line (IC_{50} 46 μM), with no detectable activity toward *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, or *Candida albicans*. The analogues tyramine and dibromotyramine were inactive in all assays, as was **2**. Brominated β-phenethylamines have been shown to have some ecologically relevant activities.[14−16] Preliminary ecological evaluation of **1** and **2** failed to detect any feeding deterrence against either the echinoderms *Patiriella regularis* and *Evechinus chloroticus* or the fishes *Forsterygion varium* and *Acanthoclinus quadridactylus*, at either the isolated or elevated levels.[17]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, and the solvent signal was used as reference. MS were recorded on a VG-7070 mass spectrometer. Analytical reversed-phase HPLC was run on a Waters 600 HPLC photodiode array system using an Alltech C18 column (5μ Econosphere, 4.6 × 150 mm) and eluting with a linear gradient of H_2O (0.05% TFA) through to MeCN. Semi-preparative C18 reversed-phase HPLC was performed with H_2O−MeOH solvent mixtures on an Alltech 10μ Econosil column (10 × 250 mm).

Animal Material. We collected the ascidian material while snorkeling (−2 m) in January 1997, and kept it frozen until used. Voucher specimens are held at the University of Auckland, Chemistry Department (97MO1−4) and at the NIWA Museum, Wellington (NZOI Stn Z9028).

* To whom correspondence should be addressed. Tel.: 64-9-373-7599 ext. 8284. Fax: 64-9-373-7422. E-mail: b.copp@auckland.ac.nz.
† University of Auckland.
‡ National Institute of Water and Atmospheric Research.

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Extraction and Isolation. The ascidians were freeze-dried (dry wt 16.97 g) and extracted with MeOH−DCM (10:1, 170 mL) for 72 h, after which the extract was filtered and the solvent removed under reduced pressure (total extract after desalting once with MeOH: 980 mg). A portion of the crude extract (213 mg) was subjected to C18 flash chromatography[18] (aqueous through to MeOH) with the 0−25% MeOH fractions containing the compounds of interest. These fractions were combined and separated on a column of Sephadex LH-20, eluting with MeOH to give **2** (fraction 3) in near purity. Compound **2** was further purified by semipreparative HPLC, MeOH−aqueous TFA (0.05%) 1:10, 5.5 mL/min yielding pure product (white solid 21 mg; 0.48% dry wt). Sephadex column fraction 4 was further purified on a reversed-phase C18 flash column (aqueous through to MeOH) with the 25−50% MeOH fraction containing impure **1**, which was further purified by semipreparative C18 HPLC, MeOH−aqueous TFA (0.05%) 1:1, 2.0 mL/min yielding **1** as the TFA salt (2.35 mg, 0.015% dry wt).

2-(3′-Bromo-4′-hydroxyphenol)ethanamine (1): UV (MeOH) λ_{max} (log ϵ) 202.7 (4.4), 282.7 (3.3) nm; IR (dry film) ν_{max} 3147 (broad), 2924, 1673, 1499, 1381, 1203, 1177, 1131, 838, 815, 800, 722 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 7.39 (1H, d, J = 2.1 Hz, H-2′, HMBC C-2, C-4′, C-3′, C-6′), 7.07 (1H, dd, J = 8.3, 2.1 Hz, H-6′, HMBC C-2, C-4′, C-2′, C-5′), 6.87 (1H, d, J = 8.3 Hz, H-5′, HMBC C-3′, C-1′), 3.11 (2H, t, J = 7.7 Hz, H-1, HMBC C-2, C-1′), 2.84 (2H, t, J = 7.7 Hz, H-2, HMBC C-1, C-2′, C-6′); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 154.69 (0, C-4′), 134.34 (1, C-2′), 130.29 (0, C-1′), 130.03 (1, C-6′), 117.65 (1, C-5′), 111.13 (0, C-3′), 41.99 (2, C-1), 33.33 (2, C-2); DEIMS m/z $[M]^+$ 217 (15), 215 (15), 188 (60), 187 (30), 186 (60), 185 (30), 135 (10), 107 (40), 105 (25), 91 (10), 78 (40), 77 (90), 63 (30), 51 (100), 46 (60), 39 (30); HREIMS m/z 216.9917 (calcd for $C_8H_{10}NO^{81}Br$, 216.9925), 214.9944 (calcd for $C_8H_{10}NO^{79}Br$, 214.9946).

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References and Notes

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