Dihydrotubastrines: Phenethylguanidine Analogues from the Indo-Pacific Marine Sponge *Petrosia* cf. *contignata*

Sam Sperry and Phillip Crews*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, California 95064

Received February 23, 1998

Two phenethylguanidine derivatives, 7,8-dihydrotubastrine (**1**) and 4-deoxy-7,8-dihydrotubastrine (**2**), along with the sterol xestobergsterol A (**3**), were isolated from the marine sponge *Petrosia* cf. *contignata*. The structures of the new natural products **1** and **2** were based on spectroscopic data and comparison to the literature properties for semisynthetic **1**. This is the first example from this compound class with a saturated acyclic C2 unit.

Sponges of the family Petrosiidae (order Haplosclerida) are particularly well represented in many Indo-Pacific coral reefs. Also, its members are of continuing interest to us because they have provided a wealth of nitrogenous compounds: the xestocyclamines,[1] the xestoaminols,[2] and (−)-halicyclamine B[3] from *Xestospongia* spp. and two manzamine analogues[4] from *Petrosia contignata*. During an expedition to the Milne Bay region of Papua New Guinea, we located a unique colony of *P.* cf. *contignata*, which was infested with numerous zoanthids. The chemical profiles of this genus are reasonably well understood and include, in addition to alkaloids, compounds such as fatty acids, sterols, and polyacetylenes.[5] However, chemical investigation of this zoanthid-containing material proved to be worthwhile, and in this report we describe the isolation of the new phenethylguanidine derivatives, 7,8-dihydrotubastrine (**1**) and 4-deoxy-7,8-dihydrotubastrine (**2**), plus the known C-15 ketosterol, xestobergsterol A (**3**).

The 2-BuOH-soluble portion of the MeOH extract of *P.* cf. *contignata* exhibited a lowfield NMR pattern inconsistent with previously published *P. contignata* chemistry. Subjecting this extract to high-speed countercurrent chromatography (HSCCC) in an EtOAc−aqueous MeOH biphasic solvent system yielded 10 fractions. The second fraction contained a mixture of equal parts of **1** and **2** (ca. 325 mg), and the eighth fraction contained nearly pure **3** (14 mg). After obtaining the ^{1}H and ^{13}C NMR spectra of the second fraction, we first assumed the presence of a single C17 structure, but FABMS analysis revealed ions for both **1** (*m*/*z* 180) and **2** (*m*/*z* 196). The presence of **3**, quickly recognized by its spectroscopic properties, was unsurprising, as it has previously been encountered by both us[6] and others[7] from sponges of the family Petrosiidae.

After HPLC purification (10% aqueous MeOH) of mixture **1**/**2**, identification of **1** was conducted in a concise fashion. The molecular formula, $C_9H_{13}N_3O_2$, was established in a stepwise fashion beginning with ESIMS data (a positive ion of 196 $[M + H]^+$ and negative ion of 194 $[M - H]^-$). NMR data contributed a partial count of C_9H_7, which directed our attention to the HRFABMS peak at *m*/*z* 196.1086 $[M + H]^+$. The ^{13}C and ^{1}H NMR data (1D and 2D) allowed identification of three substructures consisting of a trisubstituted aromatic ring, a two-carbon saturated side chain off aryl C-1, and a remaining sp^2 carbon (C-10) as part of a guanidine that was removed from the remaining skeleton (C-1 through C-8) by one nitrogen. These structures were combined as shown in **1**, and a literature search revealed that semisynthetic **1** had already been prepared from the soft coral metabolite tubastrine (**4**, from *Tubastrea aurea*).[8]

Having unveiled the identity of **1**, a parallel approach to characterize **2** was straightforward. The molecular formula of $C_9H_{13}N_2O_2$ was established by the HR-FABMS *m*/*z* of 180.1137 $[M + H]^+$. The combination of molecular formula and 2D NMR data indicated that **2** was simply a deoxygenated analogue of **1**.

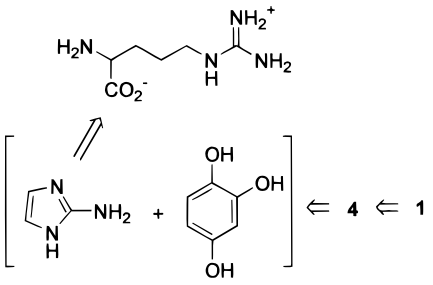
Our isolation work on the 2-BuOH extract was guided by a brine shrimp lethality assay.[9] Inexplicably, the apparent toxicity vanished after the final HPLC purification step. Both **1** and **2** were inactive toward *Staphylococcus aureus* and *Bacillus subtilis* in a disk diffusion assay, which is notable because **5** displayed weak to moderate inhibition in similar assays.

The 7,8-dihydrostyryl moiety present in structures **1** and **2** represents an unprecedented variation for the marine styryl compound class, and these compounds are quite dissimilar to all *Petrosia* (order Haplosclerida) chemistry hitherto published. To the best of our knowledge, the isolation of 2-aminoimidazole from *Reneira cratera* is the only example of a Haplosclerida-derived guanidine,[10] and there is supporting evidence that it is arginine derived.[11] Furthermore, there have been multiple reports of hydroxyhydroquinone from the sponge genus *Axinella*.[10,12] These prior findings, together with other possible precursors reported from marine organisms, suggest a biogenetic pathway to compound **1** as outlined in Scheme 1. In addition to **4**,[8] the other marine metabolite that most resembles **1** and **2** is the styryl guanidine **5** from the sponge *Spongosorites* sp.[13] Other relatives would include the sponge-derived styryl sulfates isojaspin (**6**),[14] (*E*)-narain (**7**), and (*Z*)-narain (**8**),[15] all from *Jaspis* spp. Finally, it is important to note that sterols containing the C-15 ketone and *cis* C/D ring junction such as that found in **3** are rarely encountered, especially from organisms other than sponges.[16] The other examples include only

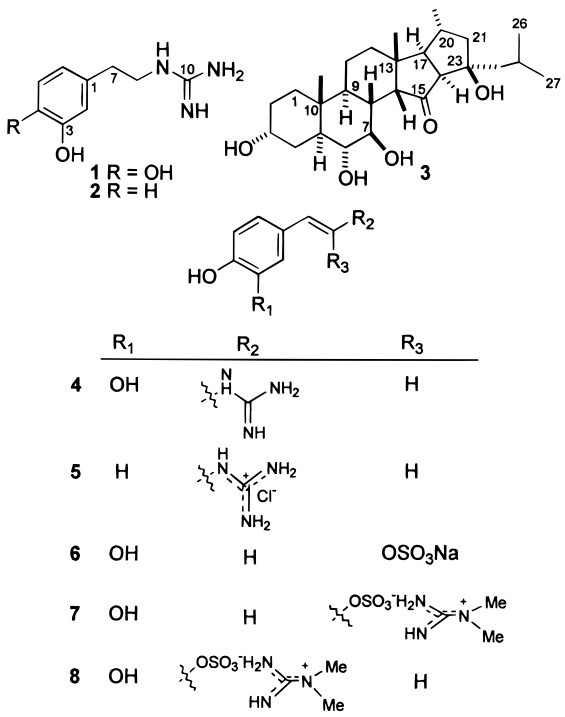
* To whom correspondence should be addressed. Tel.: (408) 459-2603. Fax: (408) 459-2935. E-mail: phil@chemistry.ucsc.edu.

S0163-3864(98)00061-5 CCC: $15.00
Published on Web 05/22/1998

Scheme 1



contignasterol, also from *Petrosia contignata*;[17] haliclostanone sulfate from *Haliclona* sp.;[18] xestobergsterols A and B from *Xestospongia berquistia*;[7a] and xestobergsterols A−C from *Ircinia* sp.[7b]



Experimental Section

General Experimental Procedures. NMR spectra were recorded at 250 or 500 MHz for ^{1}H NMR and 62.9 and 125.7 MHz for ^{13}C NMR. Multiplicities of ^{13}C NMR were determined from APT/DEPT data or HMQC (500 MHz). MS data were obtained on a VG ZAB−SE (LRFABMS), a VG 70-SE−4F (HRFABMS), and a VG Quattro (LRESIMS). Chromatography was performed using a P. C., Inc. Ito Multi-Layer Coil Separator-Extractor (HSCCC) and with ODS (reversed-phase HPLC). IR spectra were measured on a Varian 1600 series FTIR spectrometer, UV spectra were measured on a Hewlett−Packard 8452A Diode-Array spectrometer, and optical rotation was measured on a JASCO DIP-370 digital polarimeter.

Collection and Identification. The sponge (coll. no. 90167) was collected from Milne Bay, Papua New Guinea, using scuba from a depth of approximately 20 ft. and was identified by Dr. M. C. Diaz as *Petrosia* cf. *contignata* (Petrosiidae, Haplosclerida). The specimen was thick-encrusting in shape (3 cm in thickness), and of a hard consistency. Alive, its color was purplish brown externally and tan internally. Its surface was smooth but covered regularly by zooanthids, and it was sticky due to the release of a mucous substance. Oscules, round and compound (3−7 mm in diameter), are regularly distributed over the surface. The skeleton consists of a compact isotropic polyspicular reticulation (50−150 m in diameter), composed mostly of rectangular meshes. The ectosome presents a distinct tangential reticulation of thinner polyspicular tracts (30−50 m in diameter). The spicules are strongyloxea in three size classes with a range of their length and width of: (I) 200−240 × 10−15, (II) 130−170 × 8−10, (III) 60−90 × 6−8. This specimen conforms very closely to the description of *Petrosia contignata* (Thiele, 1903), differing only in its encrusting habit rather than the predominant erect ramifying branches of the species. Until the intraspecific variation of shape for this species is established we will refer to the encrusting forms as *Petrosia* cf. *contignata*. A voucher, as well as a topside photograph, are in the UCSC sponge collection archives and are available from P. C.

Extraction and Isolation. The sponge (wet wt 0.45 kg) was preserved in the field by our standard procedures,[4] and then returned to our home lab at UCSC. The organism was then soaked in MeOH for 24 h, and the solution was decanted. This procedure was repeated two more times, and the combined MeOH extracts were concentrated. The residue was subjected to sequential trituration, first with CH_2Cl_2 and then with 2-BuOH. The 2-BuOH extract (0.77 g) was chosen for further purification based on spectroscopic and mild brine shrimp toxicity. Initial fractionation was performed by HSCCC using an EtOAc−MeOH−H_2O (5:1:4) biphasic solvent system. A portion of the extract (0.5 g) was loaded onto a 2.4-mL injection loop and partitioned on a 400-mL column at 750−800 rpm with a mobile solvent flow rate of 8 mL/min. The first 120 min was operated in the reversed-phase (aqueous:mobile; organic:stationary) and seven fractions were collected (C1−C7). For an additional 60 min the system was operated in the normal phase (organic:mobile; aqueous:stationary), and three more fractions were collected (C8−C10). Fraction C2 (approx 325 mg; accurate weight measurements were difficult due to a hygroscopic nature) was determined to be an equal mixture of components **1** and **2**, which were subsequently resolved by reversed-phase HPLC (10% aqueous MeOH). Fraction C8 (6.1 mg) eluted from the HSCCC column as pure **3**.

7,8-Dihydrotubastrine (1): brown viscous oil; 6.6 mg isolated; UV (MeOH) λ_{max} (ϵ) 284 (1900), 222 (3900); IR (neat) ν 3356, 3274, 3182, 1659, 1523, 1441, 1359, 1283, 1252, 1196, 1114 cm^{-1}; ^{13}C NMR (CD_3OD, 125.7 MHz) δ 158.8 (C-10), 146.7 (C-3), 145.4 (C-4), 130.8 (C-1), 121.2 (C-6), 117.1 (C-2), 116.7 (C-5), 44.1 (C-8), 35.5 (C-7); ^{1}H NMR (CD_3OD, 500 MHz) δ 6.70 (1H, d, J = 8.0 Hz, H-5), 6.67 (1H, t, J = 2.0 Hz, H-2), 6.55 (1H, dd, J = 8.0, 2.0 Hz, H-6), 3.37 (2H, t, J = 7.0 Hz, H_2-8), 2.72 (2H, t, J = 7.0 Hz, H_2-7); H NMR (DMSO-d_6, 500 MHz) δ 8.80 (2H, br s, 3-OH, 4-OH), 7.47 (1H, br s, NH-9), 6.66 (1H, d, J = 8.0 Hz, H-5), 6.63 (1H, d, J = 1.0 Hz, H-2), 6.49 (1H, dd, J = 8.0, 1 Hz, H-6), 3.26 (2H, q, J = 7.0 Hz, H_2-8), 2.59 (2H, t, J = 7.0 Hz, H_2-7); all assignments are supported by HMQC, APT, COSY, and HMBC data; HRFABMS m/z [M + H]$^+$ 196.1086 (calcd for $C_9H_{14}N_3O_2$, 196.1086).

4-Deoxy-7,8-dihydrotubastrine (2): brown viscous oil; 3.7 mg isolated; UV (MeOH) λ_{max} (ϵ) 274 (1400), 214 (4700); IR (neat) ν 3356, 3274, 3172, 1661, 1456, 1354, 1272, 1242, 1160, 1022, 996 cm^{-1}; ^{13}C NMR (CD_3OD, 125.7 MHz) δ 159.0 (C-3, C-10), 140.9 (C-1), 130.9 (C-5), 121.1 (C-2), 116.9 (C-6), 114.8 (C-4), 43.9 (C-8), 36.0 (C-7); ^1H NMR (CD_3OD, 500 MHz) δ 7.12 (1H, t, J = 7.5 Hz, H-5), 6.71 (1H, br d, J = 7.5 Hz, H-6), 6.67 (1H, t, J = 2.3 Hz, H-6), 6.66 (1H, ddd, J = 7.5, 2.3, 1.0 Hz, H-4) 3.42 (2H, t, J = 7.0 Hz, H_2-8), 2.80 (2H, t, J = 7.0 Hz, H_2-7); ^1H NMR (DMSO-d_6, 500 MHz) δ 9.36 (1H, br s, 3-OH), 7.60 (1H, br s, NH-9), 7.09 (1H, t, J = 8.0 Hz, H-5), 6.67 (1H, d, J = 8.0 Hz, H-2), 6.66 (1H, d, J = 2.0 Hz, H-2), 6.63 (1H, dd, J = 8.0, 2.0 Hz, H-4), 3.33 (under HOD signal, H_2-8), 2.69 (2H, t, J = 7.5 Hz, H_2-7); all assignments are supported by HMQC, APT, COSY, and HMBC data; HRFABMS m/z $[M + H]^+$ 180.1137 (calcd for $C_9H_{14}N_3O_1$, 180.1137).

Xestobergsterol A (3): yellow oil; 14.4 mg; $[\alpha]_D$ −64.7° (*c* 1.1, MeOH); ^{13}C NMR (CD_3OD, 62.5 MHz) δ 219.4 s (C-15), 83.4 s (C-23), 75.6 d (C-6), 75.1 d (C-7), 66.4 d (C-3), 63.4 d (C-16), 58.5 d (C-17), 52.4 d (C-14), 52.1 t/51.9 t (C-22/C-24), 47.0 d (C-9), 42.8 d (C-5), 39.6 d (C-8), 39.2 t (C-12), 39.0 s (C-13), 37.2 s (C-10), 35.7 d (C-20), 33.3 t (C-1), 30.9 t (C-4), 28.8 t (C-2), 25.5 d (C-25), 25.2 q/24.7 q (C-26/C-27), 21.7 t (C-11), 20.8 q (C-21), 19.8 q (C-18), 12.5 q (C-19); ^1H NMR (CD_3OD, 250 MHz) 4.32 (1H, dd, J = 11, 9.0 Hz, H-7), 3.98 (1H, br s, H-3), 2.99 (1H, dd, J = 11, 9.0 Hz, H-6), 2.72 (1H, br s, H-14), 2.50 (1H, d, J = 10 Hz, H-16), 2.35 (1H, br m, H-20), 1.10 (3H, s, Me-18), 1.07 (3H, d, J = 6.3 Hz, Me-21), 0.92 (3H, d, J = 6.6 Hz, Me-26), 0.90 (3H, d, J = 6.5 Hz, Me-27), 0.75 (3H, s, Me-19); NMR data obtained for **3** in pyridine-d_5 matched well with those previously reported,[7] and these values, along with DEPT135 and DEPT90 data, were used to establish CD_3OD assignments.

Acknowledgment. Financial support at UCSC was from NIH grant CA47135. We appreciated assistance in the collection of sponges by Ms. Lisa Hunter, Dr. M. Cristina Diaz, and Dr. Larry V. Manes. Taxonomic analysis was conducted by Dr. M. Cristina Diaz (UCSC, Institute for Marine Sciences). Funds from the Elsa Pardee Foundation and NSF-CHE-93-22464 supported the purchase of a 500 MHz NMR, and from the Keck Foundation supported the purchase of an ESIMS.

References and Notes

(1) (a) Rodríguez, J.; Crews, P. *Tetrahedron Lett.* **1994**, *35*, 4719−4722. (b) Rodríguez, J.; Peters, B. M.; Kurz, L.; Schatzman, R. C.; McCarley, D.; Lou, L.; Crews, P. *J. Am. Chem. Soc.* **1993**, *115*, 10436−10437.
(2) Jiménez, C.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 978−982.
(3) Harrison, B.; Talapatra, S.; Lobkovsky, E.; Clardy, J.; Crews, P. *Tetrahedron Lett.* **1996**, *37*, 9151−9154.
(4) Crews, P.; Cheng, X.-C.; Adamczeski, M.; Rodríguez, J.; Jaspars, M.; Schmitz, F. J.; Traeger, S. C.; Pordesimo, E. O. *Tetrahedron* **1994**, *50*, 13567−13574.
(5) Nonalkaloid examples from each class include: (a) fatty acids: Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Helv. Chim. Acta* **1993**, *76*, 2814−2816. (b) sterols: see Burgoyne et al.[17] (c) polyacetylenes: Guo, Y.; Gavagnin, M.; Trivellone, E.; Cimino, G. *J. Nat. Prod.* **1995**, *58*, 712−722.
(6) Author's unpublished results.
(7) (a) Shoji, N.; Umeyama, A.; Shin, K.; Katsuko, T.; Arihara, S.; Kobayashi, J.; Takei, M. *J. Org. Chem.* **1992**, *57*, 2996−2997. (b) Kobayashi, J.; Shinonaga, H.; Shigemori, H.; Umeyama, A.; Shoji, N.; Arihara, S. *J. Nat. Prod.* **1995**, *58*, 312−318. (c) Synthesis: Jung, M. E.; Johnson, T. W. *J. Am. Chem. Soc.* **1997**, *119*, 12412−12413.
(8) Sakai, R.; Higa, T. *Chem. Lett.* **1987**, 127−128.
(9) Anderson, J. E.; Goetz, C. M.; McLaughlin, J. L.; Suffness, M. *Phytochemical Anal.* **1991**, *2*, 107−111.
(10) Cimino, G.; De Stefano, S.; Minale, L. *Comp. Biochem. Physiol.* **1974**, *47B*, 895−897.
(11) Seki, Y.; Nakamura, T.; Okami, Y. *J. Biochem.* **1970**, *67*, 389−396.
(12) Wratten, S. J.; Meinwald, J. *Experientia* **1981**, *37*, 13−14.
(13) Urban, S.; Capon, R. J.; Hooper, J. N. A. *Aust. J. Chem.* **1994**, *47*, 2279−2282.
(14) Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett.* **1994**, *35*, 4579−4580.
(15) Tsukamoto, S.; Kato, H.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 5873−5874.
(16) One possible example of a *trans* C/D ring junction for a C15 keto-steroid is taccalanolide M from *Tacca plantaginea* (Shen, J.; Chen, Z.; Gao, Y. *Phytochemistry* **1996**, *42*, 891−893) but see discussion in the Ph.D. dissertation of S.S.: Sperry, S. Ph.D. Dissertation, University of California, Santa Cruz, 1998, Chapter 4.
(17) Burgoyne, D. L.; Andersen, R. J.; Allen, T. M. *J. Org. Chem.* **1992**, *57*, 525−528.
(18) Sperry, S.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 29−32.

NP980061Z