Euthyroideones, Novel Brominated Quinone Methides from the Bryozoan *Euthyroides episcopalis*

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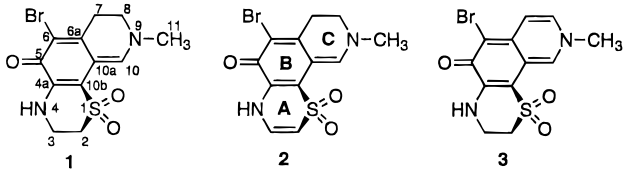
Although comparatively little research has been undertaken into bryozoan secondary metabolites as compared with those of other marine invertebrates, bryozoans have proven to be an excellent source of novel and/or biologically active compounds.[1] The most well known of these metabolites are the bryostatins,[2] but other examples include the tambjamines,[3a,b] the securamines,[4a,b] the amathamides,[5a−c] and the amathaspiramides.[6] The majority of bryozoan metabolites isolated to date have been alkaloids.

In our continuing survey of the chemical composition of New Zealand marine bryozoans, we undertook an investigation of an extract of *Euthyroides episcopalis* (order Cheilostomatida, suborder Ascophorina, family Euthyroididae). We report here the isolation and structural elucidation of three novel alkaloids, euthyroideones A−C (**1**−**3**) from the bryozoan. An X-ray crystal structure of euthyroideone A (**1**) was obtained, and the biological activity of the alkaloids was examined.



E. episcopalis is endemic to the Australasian region, and its New Zealand distribution is from Napier to Stewart Island.[7] The bryozoan was collected by SCUBA from Fiordland, off the South Island of New Zealand, and identified as *E. episcopalis*. Frozen bryozoan was extracted with $MeOH/CH_2Cl_2$ (3:1). The extract was subjected to reversed-phase flash column chromatography, gel permeation chromatography, and HPLC to give the euthyroideones A−C (**1**−**3**) in 0.0004, 0.0005, and 0.0003% yield, respectively (based on bryozoan wet weight).

HREIMS of euthyroideone A (**1**) exhibited a pair of molecular ion peaks at *m*/*z* 345.981 40 and 343.982 67, consistent with a molecular formula of $C_{12}H_{13}BrN_2O_3S$. The ^{1}H NMR spectrum of **1** (Table 1) in acetone-d_6/D_2O contained four methylene resonances and a sharp single proton resonance at 8.55 ppm. The chemical shift of an N-methyl signal at 3.68 ppm, which was obscured by the HOD peak, was confirmed from correlations observed in the HMBC spectrum of **1** (Table 1). A ^{1}H NMR spectrum was also acquired in pure acetone-d_6. Solubility was poor in this solvent, but the spectrum contained an additional broad, one-proton signal at 6.33 ppm. Also, one of the methylene signals was now a complex multiplet, indicating additional coupling from the exchangeable proton. The ^{13}C NMR spectrum acquired in acetone-d_6/D_2O (Table 2) contained 12 resonances: four aliphatic methylene signals, one aromatic methine signal, one N-methyl signal, and six quaternary carbon signals. Atom connectivities were established by HMQC and HMBC NMR experiments.

The complete structure of euthyroideone A (**1**) was deduced with the aid of a single-crystal X-ray diffraction study. Suitable crystals of **1** were obtained from water by slow evaporation. An ORTEP plot of the crystal structure of euthyroideone A (**1**) is shown in Figure 1. The crystal structure reveals a pyrido[4,3-*h*]-1,4-benzothiazine skeleton with amine nitrogens at positions 4 and 9, a sulfone group at position 1, and a bromine substituent at position 6. Conjugation extends from the central chromophore to the enamine nitrogens N4 and N9, with the N4−C4a and N9−C10 bonds showing double bond character (bond lengths of 1.35 and 1.32 Å, respectively). The sulfur and bromine atoms are located 0.35 and 0.14 Å, respectively, above the root-mean-square (RMS) plane of the central ring, resulting in C10b and C6 also being slightly above this plane. There is considerable strain in ring A to accommodate the sulfur atom, with C10b−C4a−N4 and C4a−N4−C3 angles of 126° and a C10b−S1−C2 angle of 101°.

The ^{1}H NMR spectrum of euthyroideone B (**2**) in acetone-d_6 resembled that of euthyroideone A (**1**) but contained only two triplets representative of two methylene protons each and two methine proton signals indicative of a double bond, a doublet, and a doublet of doublets, with the latter collapsing to a simple doublet on addition of D_2O (Table 1). The ^{13}C NMR spectrum of **2** in acetone-d_6/D_2O (Table 2) also resembled that of **1** in most respects, but of the 12 resonances it contained, there were two aliphatic methylene carbon signals, three aromatic methine carbon signals, one N-methyl signal, and six quaternary carbon signals. These spectra implied that the structure of **2** was very similar to that of **1** but that it contained a double bond between C2 and C3. HMBC and HMQC NMR experiments facilitated assignment of all ^{1}H and ^{13}C NMR spectral signals, and HREIMS established a molecular formula of $C_{12}H_{11}BrN_2O_3S$.

The ^{1}H NMR spectrum of euthyroideone C (**3**) (Table 1) was similar to that of euthyroideone B (**2**) in that it contained three aromatic proton signals, two methylene multiplets, and one N-methyl resonance. However, the aromatic proton signals were closer together and at lower field than in the spectrum of **2**, and the N-methyl signal resonated at an unusually low field at 4.39 ppm. Eu-

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Table 1. ^{1}H NMR Data (δ in ppm, J in hertz) for Euthyroideones A−C (1−3) in Acetone-d_6/D_2O

	1		2		3	
proton		HMBC (H to C)		HMBC (H to C)		HMBC (H to C)
2	3.51 m	C3	5.95 d (8.6)	C10b	3.64 m	C3
3	4.03 m	C2, C4a	7.47 d (8.6)	C2, C4a	4.19 m	C2, C4a
4	6.33[a] br s					
7	3.24 t (8.2)	C6, C6a, C8, C10a	3.35 t (8.1)	C6, C6a, C8, C10a	7.85 d (7.0)	C6, C8, C10a
8	3.87 t (8.2)	C6a, C7, C10, C11	3.99 t (8.1)	C6a, C7, C10, C11	8.12 dd (7.0, 1.4)	C6a, C7, C11
10	8.55 s	C6a, C8, C10a, C10b, C11	8.95 s	C6a, C8, C10a, C10b, C11	9.03 d (1.4)	C6a, C8, C10a, C10b, C11
11	3.68 s	C8, C10	3.76 s	C8, C10	4.39 s	C8, C10

[a] Chemical shift obtained in acetone-d_6.

Table 2. ^{13}C NMR Data (δ in ppm) for Euthyroideones A−C (1−3)

	acetone-d_6/D_2O		DMSO-d_6
carbon	1	2	3
2	51.4	98.7	48.4
3	39.9	134.1	38.6
4a	138.7	130.2	143.5
5	167.8	166.8	162.7
6	113.9	114.4	100.4
6a	136.0	139.4	134.0
7	29.5	29.3	115.8
8	48.2	48.1	133.1
10	153.7	155.3	133.7
10a	102.2	100.2	113.8
10b	114.9	121.6	104.5
11	45.8	46.4	45.5

Table 3. P388 and Antiviral/Cytotoxicity Assay Results for Euthyroideones A−C (1−3)

compound	P388 IC_{50}[a]	HSV[b]	PV1[c]	Cyt[d]
1	>12 500	−[e]	−	−
2	>12 500	?	?	1+
3	>12 500	−	−	−

[a] The concentration of sample in ng/mL required to reduce the cell growth of the P388 leukemia cell line (ATCC CCL 46) by 50%. [b] *Herpes simplex* type 1 (strain F, ATCC VR 733) virus grown on the BSC-1 cell line (ATCC CCL 26). [c] *Polio virus* (Pfizer vaccine strain) grown on the BSC-1 cell line. [d] Cytotoxicity to BSC-1 cells at 60 μg/disc. [e] Key: −, no discernable antiviral or cytotoxic effects; ?, indeterminate activity; 1+, antiviral/cytotoxic zone 1−2 mm excess radius from disc edge.

thyroideone C (**3**) was only sparingly soluble in most organic solvents, but the ^{13}C NMR spectrum was finally acquired in DMSO-d_6 (Table 2). The spectrum again contained 12 resonances and was similar to that of **1**, but the chemical shifts of carbons in ring C were now indicative of the presence of a pyridine ring. HREIMS established a molecular formula of $C_{12}H_{11}BrN_2O_3S$, confirming that euthyroideone C (**3**) is the 7,8-dehydro analogue of euthyroideone A.

A mixture of euthyroideones A (**1**) and B (**2**) was assayed in P388 and antimicrobial assay systems. No activity was observed in either assay. Due to a lack of material, pure euthyroideones A−C (**1**−**3**) were therefore assayed in P388 murine leukemia and antiviral/cytotoxicity assay systems only. None of the compounds exhibited any significant activity in the assays, except euthyroideone B (**2**), which was weakly cytotoxic to the BSC-1 cell line (derived from African Green Monkey kidney cells) used in the antiviral/cytotoxicity assay (Table 3).

Other marine-derived polycyclic quinones that possess the same amine/sulfone substructure as in ring A of the euthyroideones have been isolated from sponges. The adociaquinones were isolated from the sponge *Adocia* sp. from Truk[8] and xestoquinolide B was isolated from a Vanuatu collection of the sponge *Xestospongia* cf. *carbonaria*.[9] The euthyroideones, however, possess a unique heterocyclic ring system that contains brominated quinone methide, sulfone, and amine groups.

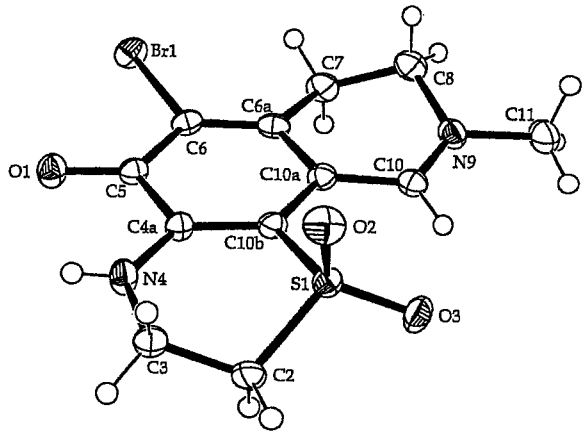

Figure 1. ORTEP diagram of euthyroideone A (**1**).

Experimental Section

General Experimental Procedures. NMR spectra were determined on a 9.4 T instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The ^{13}C NMR spectrum of euthyroideone C was determined on a 14.1 T instrument at 150 MHz. ^{1}H and ^{13}C NMR chemical shifts are referenced in acetone-d_6 to the solvent (2.20 and 30.20 ppm respectively), and ^{13}C NMR chemical shifts are referenced in DMSO-d_6 to the solvent at 39.50 ppm. Heteronuclear ^{1}H−^{13}C connectivities were determined by HMQC and HMBC experiments. High-resolution mass spectral data were obtained in the EI mode.

Collection of *E. episcopalis*. Colonies of bryozoans (1200 g wet weight) were collected by SCUBA in 1989 from Fiordland, New Zealand, and stored frozen. A voucher specimen, 89-FL10-08, is held at the Department of Chemistry, University of Waikato. The bryozoan was identified by Dr. D. P. Gordon. The zooids of the bryozoan were arranged in unilamellar sheets giving compressed frondose branches 2−3 mm wide. Colonies were up to 10 cm high and were a dull brownish-yellow color.

Isolation and Characterization. The bryozoan (200 g wet weight) was macerated in a blender and extracted with MeOH/CH_2Cl_2 (3:1, 800 mL) three times. The combined extract was filtered, and the solvent was removed in vacuo. The crude extract (7 g) was fractionated by reversed-phase flash column chromatography on C_{18} silica using a steep-stepped gradient from H_2O to MeOH to CH_2Cl_2.[10] A MeOH soluble fraction from this column (98.5 mg) contained a bright yellow, fluorescent spot by TLC (silica, EtOAc:acetone (1:1)). This fraction was subjected to gel permeation on Sephadex LH-20 in MeOH to yield a late eluting, yellow fraction (4.3 mg). HPLC of this fraction (Alltech

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Econosil C_{18}, MeOH/water (1:1), flowrate 5 mL/min, UV detection at 254 and 350 nm) yielded euthyroideones A (**1**) (0.7 mg), euthyroideone B (**2**) (0.9 mg), and euthyroideone C (**3**) (0.5 mg).

Euthyroideone A (1): mp 245 °C; UV (MeOH) λ_{max} (ϵ) 202 (3.96), 242 (3.72), 383 (3.60), 452 (3.86); IR ν_{max} (KBr disk) 3383, 1632, 1560, 1495, 1415, 1385, 1328, 1279, 1217, 1119, 1074 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m*/*z* (%) (+20 V) 347, 345 (100, 98), 283, 281 (41, 50); HREIMS *m*/*z* found 345.981 40 (calcd for $C_{12}H_{13}{}^{81}BrN_2O_3S$, 345.980 98), 343.982 67 (calcd for $C_{12}H_{13}{}^{79}BrN_2O_3S$, 343.983 03).

Euthyroideone B (2): mp 244 °C; UV (MeOH) λ_{max} (ϵ) 208 (4.50), 310 (4.38), 436 (4.60); IR ν_{max} (KBr disk) 3424, 1636, 1561, 1503, 1417, 1383, 1361, 1222, 1113, 1028 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m*/*z* (%) (+20 V) 345, 343 (100, 95); HREIMS *m*/*z* found 343.966 95 (calcd for $C_{12}H_{11}{}^{81}BrN_2O_3S$, 343.965 33), 341.967 47 (calcd for $C_{12}H_{11}{}^{79}BrN_2O_3S$, 341.967 38).

Euthyroideone C (3): UV (MeOH) λ_{max} (ϵ) 203 (3.60), 280 (3.63), 391 (3.32); IR ν_{max} (KBr disk) 3425, 1636, 1546, 1490, 1384, 1275, 1121, 1027 cm^{-1}; for ^1H and ^{13}C NMR, see Tables 1 and 2; ESMS *m*/*z* (%) (+20 V) 345, 343 (97, 100), 281, 279 (26, 44); HREIMS *m*/*z* found 343.971 21 (calcd for $C_{12}H_{11}{}^{81}BrN_2O_3S$, 343.965 33), 341.967 53 (calcd for $C_{12}H_{11}{}^{79}BrN_2O_3S$, 341.967 38).

X-ray Crystallography of Euthyroideone A (1). The unit cell dimensions and intensity data were obtained on a Siemens SMART diffractometer. The data collection nominally covered over a hemisphere of reciprocal space, by a combination of three sets of exposures. Each set had a different ϕ angle for the crystal, and each exposure covered 0.3° in ω. The crystal-to-detector distance was 5.0 cm. The data sets were corrected empirically for absorption using SADABS.[11]

The structure was solved by direct methods using the SHELXS-86 structure-solving package[12] and refined with the SHELXL-93 package[13] using full-matrix least squares based on F^2. All non-hydrogen atoms were treated anisotropically, and hydrogen atoms were included in their calculated positions except for H(1) on N(1), which was located in a penultimate difference map and refined with an isotropic temperature factor.

Crystal Data: $C_{12}H_{13}BrN_2O_3S$, M_r = 345.21; monoclinic, space group $P2_1/c$ with a = 5.7860(1), b = 22.9564(3), c = 9.8121-(1) Å, β = 103.428(1)°, V = 1267.67(3), Z = 4, d_c = 1.809 g cm^{-3}, F(000) 696, μ (Mo Kα) = 3.413 mm^{-1}; crystal size 0.58 × 0.19 × 0.12 mm^3. A total of 7956 reflections were collected at 203 K in the range 1.8° < θ < 28.2°, corresponding to 2932 unique data (R_{int} = 0.0196), $T_{max,min}$ 0.752,0.552. The refinement converged with $R1$ = 0.0285 (for 2542 data with $I > 2\sigma(I)$), R_1 = 0.0355, wR_2 = 0.0661, GoF 1.102 (all data). The largest features in a final difference map were +0.418/−0.363 e $Å^{-3}$.

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Supporting Information Available: ^1H spectra for compounds **1**−**3**, tables of crystal data, bond lengths and angles, atomic coordinates, and anisotropic thermal parameters (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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