The Isolation and Synthesis of Novel Nematocidal Dithiocyanates from an Australian Marine Sponge, *Oceanapia* sp.

Robert J. Capon,*,† Colin Skene,† Edward Hsiang-Te Liu,† Ernest Lacey,[1] Jennifer H. Gill,‡ Kirstin Heiland,§ and Thomas Friedel§

School of Chemistry, University of Melbourne, Parkville, Victoria 3010, Australia

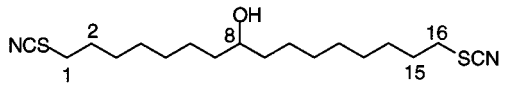
r.capon@unimelb.edu.au

Received July 5, 2001

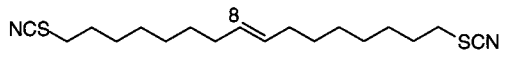
Bioassay-directed fractionation of the EtOH extract of an *Oceanapia* sp. collected off the northern Rottnest Shelf, Australia, has yielded three novel dithiocyanates, thiocyanatins A (**1**), B (**2a**), and C (**2b**). The structures were determined by detailed spectroscopic analysis and confirmed by total synthesis. In addition to featuring an unprecedented dithiocyanate functionality, thiocyanatins possess an unusual 1,16-difunctionalized *n*-hexadecane carbon skeleton and are revealed as a hitherto unknown class of nematocidal agent.

Introduction

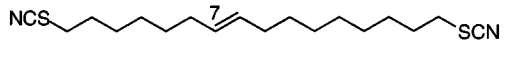
During our ongoing investigations into Australian marine metabolites as antiparasitic agents with potential application in animal health, we have encountered numerous structurally novel nematocides. These have included an extensive array of epoxylipids from the brown alga *Notheia anomala*,[1] as well as the alkaloids geodin A Mg salt,[2] the amphilactams,[3] and onnamide F[4] from various southern Australian sponges. These prior successes have encouraged a belief that marine metabolites hold considerable promise as a source for bioprospecting new-generation antiparasitics. In this paper, we extend the class of marine natural products that display promising nematocidal properties to include lipid thiocyanates. While marine metabolites incorporating a thiocyanate moiety are known, reports on such compounds do not feature prominently in the marine natural products literature. Such accounts as do exist tend to be dominated by monothiocyanate substituted terpenes from sponges.[5−11] Departures from this trend include the bromotyrosine thiocyanate psammaplin B,[12] from the sponge *Psammaplysilla purpurea*, and the alkaloid thiocyanate cylindricines F−H,[13,14] from the ascidian *Clavelina cylindrica*. Marine thiocyanates have been attributed a range of biological properties, including cytotoxicity,[6] antifouling,[7] antimalarial,[9] and antifungal[11] activity. In this paper, we describe the isolation, structural elucidation, and synthesis of three new dithiocyanates, designated thiocyanatin A (**1**), B (**2a**), and C (**2b**), from a marine sponge of the genus *Oceanapia*, collected off the northern Rottnest Shelf, Australia. In addition to featuring an unprecedented dithiocyanate functionality, thiocyanatins possess an unusual 1,16-difunctionalized *n*-hexadecane carbon skeleton and are revealed as a hitherto unknown class of nematocidal agent. Preliminary structure−activity relationship (SAR) studies have identified key aspects of the nematocidal pharmacophore and as such indicate worthwhile directions for future SAR investigations.



1



2a



2b

Results and Discussion

The crude aqueous ethanol extract of an *Oceanapia* sp. collected during scientific trawling operations off the northern Rottnest Shelf, Australia, displayed potent nematocidal activity against the commercial livestock

* To whom correspondence should be addressed. Phone: +61 3 8344 6468. Fax: +61 3 9347 5180.
† School of Chemistry, University of Melbourne, Parkville, Victoria 3010, Australia.
‡ Microbial Screening Technologies, Kemps Creek, New South Wales 2171, Australia.
§ Novartis Animal Health Australia Pty. Ltd., Kemps Creek, New South Wales 2171, Australia.

(1) Capon, R. J.; Barrow, R. A.; Rochfort, S.; Jobling, M.; Skene, C.; Lacey, E.; Gill, J.; Friedel, T.; Wadsworth, D. *Tetrahedron* **1998**, *54*, 2227−2242.
(2) Capon, R. J.; Skene, C.; Lacey, E.; Gill, J. H.; Wadsworth, D.; Friedel, T. *J. Nat. Prod.* **1999**, *62*, 1256−1259.
(3) Ovenden, S. P. B.; Capon, R. J.; Lacey, E.; Gill, J. H.; Friedel, T.; Wadsworth, D. *J. Org. Chem.* **1999**, *64*, 1140−1144.
(4) Vuong, D.; Capon, R. J.; Lacey, E.; Gill, J. H.; Heiland, K.; Friedel, T. *J. Nat. Prod.* **2001**, *64*, 640−642.
(5) He, H.; Faulkner, D. J.; Shumsky, J. S.; Hong, K.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 2511−2514.
(6) Pham, A. T.; Ichiba, T.; Yoshida, W. Y.; Scheuer, P. J.; Uchida, T.; Tanaka, J.; Higa, T. *Tetrahedron Lett.* **1991**, *32*, 4843−4846.
(7) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 9447−9454.
(8) He, H.; Salvá, J.; Catalos, R. F.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 3191−3194.
(9) Simpson, J. S.; Garson, M. J.; Hooper, J. N. A.; Cline, E. I.; Angerhofer, C. K. *Aust. J. Chem.* **1997**, *50*, 1123−1127.
(10) Simpson, J. S.; Garson, M. J. *Tetrahedron Lett.* **1998**, *39*, 5819−5822.
(11) Fusetani, N.; Wolstenholme, H. J.; Shinoda, K.; Asai, N.; Matsunaga, S.; Onuki, H.; Hirota, H. *Tetrahedron Lett.* **1992**, *33*, 6823−6826.
(12) Jiménez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097−2102.
(13) Li, C.; Blackman, A. J. *Aust. J. Chem.* **1994**, *47*, 1355−1361.
(14) Li, C.; Blackman, A. J. *Aust. J. Chem.* **1995**, *48*, 955−965.

10.1021/jo0106750 CCC: $20.00
Published on Web 10/02/2001

parastite *Haemonchus contortus* (LD_{99} = 135 μg/mL). Bioassay-directed fractionation proceeded via concentrating the decanted aqueous EtOH extract in vacuo, triturating with CH_2Cl_2, and submitting the soluble fraction to silica SPE, followed by normal-phase HPLC. This processing afforded as the sole bioactive principle thiocyanatin A (**1**) (LD_{99} = 1.3 μg/mL), together with an inseparable mixture of inactive analogues, thiocyanatins B (**2a**) and C (**2b**).

High-resolution ESI(+)MS analysis of **1** revealed a pseudomolecular ion (M + H, *m/z* 357.2022) consistent with a molecular formula ($C_{18}H_{32}N_2OS_2$, Δmmu = −1.2) requiring four double-bond equivalents (DBE). The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1** exhibited resonances consistent with a hydroxymethine (δ 3.60, m) and two symmetric deshielded methylenes (δ 2.95, t). The ^{13}C NMR (100 MHz, $CDCl_3$) data for **1** revealed 15 methylene carbons (25.4−37.4 ppm), along with a hydroxymethine (71.8 ppm) and a quaternary (112.4 ppm) resonance. The data described above required that **1** consist of an unbranched acyclic C_{16} lipid incorporating a secondary-OH and identical deshielding terminal functional groups. Each terminal functional group must incorporate the elements of SCN and account for two DBE and a deshielded quaternary carbon (112.4 ppm). The only two functional groups that satisfy these requirements are thiocyanate (−SCN) and isothiocyanate (−NCS). The IR data for **1** was supportive of this conclusion, revealing absorbances at 3500 (br) and 2155 (sh) cm^{-1} consistent with the presence of hydroxy and SCN or NCS substituents, respectively. ^{1}H and ^{13}C NMR chemical shifts for the terminal C1 and C16 methylenes unambiguously identified the terminal functional groups as thiocyanates. Connection to the more electronegative nitrogen in an isothiocyanate (−NCS) as opposed to the sulfur in a thiocyanate (−SCN) results in greater deshielding of the methylene (^{1}H δ 3.5; ^{13}C 45 ppm)[15] compared to that observed in **1** (^{1}H δ 2.95; ^{13}C 34.0 ppm).

The position of the hydroxy moiety along the C_{16} chain in **1** was determined by EIMS, which revealed intense ions at *m/z* 186 $[HO{=}CH(CH_2)_7SCN]^{\bullet+}$ and *m/z* 200 $[HO{=}CH(CH_2)_8SCN]^{\bullet+}$ consistent with cleavage either side of a C8 hydroxy. Thus, the structure of thiocyanatin A (**1**) was determined to be 1,16-dithiocyanato-8-hydroxyhexadecane. Given the almost symmetric nature of **1**, it was not surprising that no optical rotation was observed. At this stage, the stereochemical character of **1** remains unknown. This structure assignment was confirmed by the total synthesis of thiocyanatin A (**1**) in seven steps from 8-bromooctanoic acid (**3**), as described later in this paper.

High-resolution ESI(+)MS analysis of the mixture **2a/2b** revealed a pseudomolecular ion (M + H, *m/z* 339.1919) consistent with a molecular formula ($C_{18}H_{30}N_2S_2$, Δmmu = −1.0) that suggested **2a/2b** were dehydration products of **1**. Supportive of this conclusion, the ^{1}H NMR spectrum of **2a/2b** differed from **1** in that the H8 hydroxymethine multiplet was replaced by a two proton olefinic resonances (δ 5.4) and four proton allylic methylene resonances (δ 2.0). Likewise, the IR spectrum of **2a/2b** did not display the hydroxy absorbance so prominent in **1**, but rather was limited to the thiocyanate absorbance at 2155 cm^{-1}. That **2a/2b** was a two-component mixture was not immediately evident from the data described above, but was unambiguously apparent on examination of the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **2a/2b** exhibited two sets of resonances in a ratio of 2:1.

A ^{13}C NMR DEPT experiment revealed the more intense set of resonances in the ^{13}C NMR data for **2a/2b** to consist of one olefinic methine (130.2 ppm), one quaternary (112.4 ppm) and seven methylene (27.9−34.0 ppm) carbons. This implied a high degree of symmetry, and thus, thiocyanatin B (**2a**) was determined to be the symmetrical 1,16-dithiocyanato-8-hexadecene as shown. The *E* stereochemistry about $\Delta^{8,9}$ was assigned on the basis of the ^{13}C NMR chemical shift for the allylic methylene (*E* 32.6 ppm, *Z* 29.9 ppm, **2a** 32.4 ppm).[16]

The ^{13}C NMR data attributed to **2b** included distinct resonances for two olefinic methine (129.9 and 130.6 ppm) and 10 methylene (27.7 to 32.5 ppm) carbons. ^{13}C NMR resonances for terminal methylenes C1, C2, C15, and C16 and thiocyanate carbons in **2b** overlapped with those in **2a**, suggestive of greater similarity in structure at the molecular termini. This analysis was consistent with **2b** being the $\Delta^{7,8}$ double-bond regioisomer of **2a**. Supportive of this proposition was the realization that **2a/2b** could be biosynthetically related to **1** through simple dehydration. Such a transformation would be expected to yield a 1:1 mixture of alkenes, as proposed for **2a/2b**. The *E* stereochemistry about $\Delta^{7,8}$ in **2b** was assigned on the basis of the ^{13}C NMR chemical shift for the allylic methylenes (^{13}C: C6/C9: 32.3/32.5 ppm). Thus, thiocyanatin C (**2b**) was proposed to be (*E*)-1,16-dithiocyanato-7-hexadecene as shown. Confirmation of the structures for both **2a** and **2b** was secured by total synthesis.

Thiocyanatin A (**1**) was synthesized in seven steps from 8-bromooctanoic acid (**3**) as outlined in Scheme 1. Thus, commercially available 8-bromooctanoic acid (**3**) was esterified to its methyl ester **4**, followed by conversion to the known Wittig salt **5** [17] in a two-step yield of 71%. The one-pot oxidation−Wittig coupling procedure of Noiret et al.[18] was modified by bubbling oxygen into the reaction mixture, affording the olefin−diester **6** in 72% yield with an *Z*/*E* ratio[19] of 14:1. Treatment of **6** with *m*-CPBA gave the corresponding epoxide, which was fully reduced with $LiAlH_4$ to the triol **7** in a two-step yield of 61%. The triol **7** is itself a terrestrial natural product, having been isolated from the stem cutin of *Psilotum nudum*.[20] To the best of our knowledge, this current synthesis of **7** represents the first total synthesis and complete characterization of this natural product. The triol **7** was treated with 2 equiv of TsCl to give the ditosylate **8**, which was resolved from mono- and tritosylate byproducts by preparative silica gel chromatography. Displacement of the tosylate groups by thiocyanate afforded racemic thiocyanatin A ((±)-**1**) in 38% yield from the triol **7**. Synthetic (±)-**1** and natural **1** were spectroscopically identical and exhibited the same nematocidal properties.

(15) *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra Edition 1*; The Aldrich Chemical Co.: Milwaukee, 1993; Vol. 1.

(16) *Sadtler Standard Carbon-13 Indexes*; Sadtler Research Laboratories: Philadelphia, 1980.

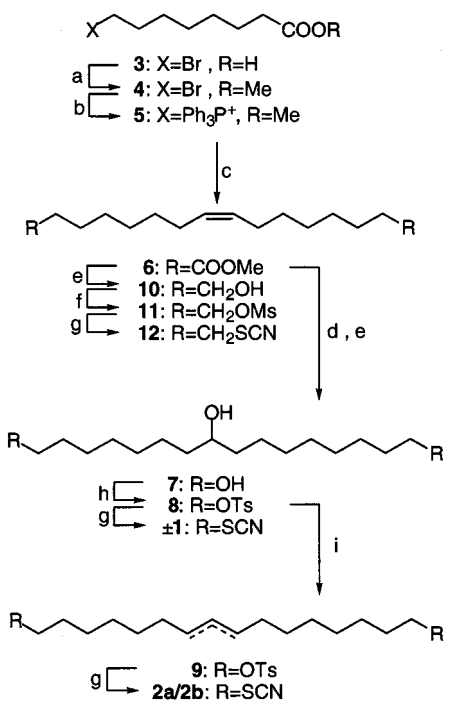
(17) Dawson, M. I.; Vasser, M. *J. Org. Chem*. **1977**, *42*, 2783−2785.

(18) Poulain, S.; Noiret, N.; Patin, H. *Tetrahedron Lett*. **1996**, *37*, 7703−7706.

(19) Determined by ^{13}C NMR peak height measurement of the olefinic carbon and comparison to literature chemical shift data (see ref 22).

(20) Caldicott, A. B.; Simoneit, B. R. T.; Eglinton, G. *Phytochemistry* **1975**, *14*, 2223−2228.

Scheme 1[a]



[a] Reagents: (a) H_2SO_4, MeOH, reflux 16 h; (b) PPh_3, MeCN, reflux 16 h; (c) NaHMDS, THF/DMPU, O_2, 60 °C, 16 h; (d) *m*-CPBA, CH_2Cl_2, rt, 16 h; (e) $LiAlH_4$, reflux 20 h; (f) MsCl, CH_2Cl_2, −10 °C, 1 h; (h) *p*-TsCl, CH_2Cl_2, DMAP/NEt_3, rt, 32 h; (g) KSCN, THF, reflux 16 h; (i) *p*-TsOH, toluene, reflux 16 h.

Treatment of the ditosylate **8** with TsOH in refluxing toluene effected nonregiospecific dehydration to a mixture of the sterically more favored (*E*)-hexadecene ditosylates **9**. Subsequent displacement of the tosylate groups with thiocyanate yielded a mixture of thiocyanatin B and C (**2a**/**2b**) in an overall yield of 46% from **8**. Synthetic **2a/2b** was spectroscopically identical to the natural mixture and, as with the natural **2a/2b**, displayed no nematocidal properties.

To provide further evidence for assignment of *E* stereochemistry in **2a/2b**, the symmetric (*Z*)-dithiocyanate **12** was prepared by reducing the (*Z*)-alkene diester **6** with $LiAlH_4$ to give the diol **10** in 86% yield. Conversion to the dimesylate **11** and subsequent displacement of the mesylate groups by thiocyanate afforded (*Z*)-1,16-dithiocyanato-8-hexadecene (**12**), the stereoisomer of thiocyanatin B (**2a**), in 57% yield from **10**. Noteworthy in the characterization of the (*Z*)-alkene **12** was the ^{13}C NMR chemical shifts of the allylic methylenes (27.0 ppm), which were significantly shielded by comparison to the natural and synthetic **2a/2b** (*E*)-alkenes (32.3−32.5 ppm), confirming prior assignment of double bond stereochemistry.

The in vitro nematocidal activities[21] against *Haemonchus contortus* for natural and synthetic **1** were found to be equipotent, while no in vitro nematocidal activity was found for either natural or synthetic **2a/2b** or for the geometric isomer **12**. From this analysis, it can be seen that the secondary-OH substituent appears necessary for nematocidal activity. Future investigations into the pharmacophore for this structure class might explore chain length, secondary-OH regioisomers, and replacement of the secondary-OH with other functional groups. A more detailed account of SAR investigations will be reported at a future date.

(21) Gill, J. H.; Redwin, J. M.; Van Wyk, J. A.; Lacey, E. *Int. J. Parasitol.* **1995**, *25*, 463−470.

Experimental Section

General Experimental Procedures. Melting points were determined using a hot-stage apparatus and are uncorrected. Rapid silica filtrations were carried out by stepwise elution, under low vacuum, using Merck 9385 silica gel loaded into a sintered glass funnel. Solid-phase extraction was carried out by using either Alltech Maxi-clean silica 900 mg cartridges or Alltech Maxi-clean C_{18} 900 mg cartridges attached to luer-lock syringes. Thin-layer chromatography was carried out using Merck silica gel 60 F_{254} sheets and visualized using both short (254 nm) and long wavelength (365 nm) ultraviolet light, as well as by dipping with 1.25% vanillin in 1:50 concentrated sulfuric acid/acetic acid solution followed by moderate heating. Chiroptical measurements ($[\alpha]_D$) were obtained on a digital polarimeter in a 100 by 2 mm cell. Ultraviolet (UV) absorption spectra were obtained using a double-beam spectrophotometer, while infrared (IR) spectra were acquired using a FT-IR spectrometer under PC control running Bio-rad Win-IR software. ^1H and ^{13}C NMR spectra as well as two-dimensional NMR experiments were performed at 400 and 100 MHz, respectively, in the solvents indicated and referenced to residual ^1H signals in the deuterated solvents. EI mass spectra were acquired at 70 eV, and high-resolution ESI mass measurements were made at a cone voltage of 100 kV. The term standard workup refers to extraction of the quenched reaction mixture with the indicated organic solvent, washing of the organic extract with water, then brine, drying of the organic extract over anhydrous $MgSO_4$ or Na_2SO_4, and removal of the organic solvent under reduced pressure.

Animal Material. The *Oceanapia* specimen (35.3 g dry weight) collected off the northern Rottnest Shelf by epibenthic sled in January 1996 was assigned the Museum of Victoria Registry No. F80009. A description is as follows: growth form, excavating base with hollow fistules (10−20 mm); color in life pink-beige, color in EtOH beige; texture compressible but harsh; oscules large, apical on fistules; surface opaque, optically smooth but minutely hispid; spicules megascleres oxeas hastate-strongylote 130−140 × 10 μm); micorscleres none; ectosome tangential spicules on the exterior surface of the fistule and a thin collagenous layer on the interior of the fistule wall; choanosome not seen, section through fistule shows a circular mesh formed by multispicular bundles of oxeas.

Isolation. The specimen was diced, steeped in EtOH, and stored at −20 °C until required. The EtOH extract was then concentrated under reduced pressure, the residue triturated with CH_2Cl_2 and the CH_2Cl_2 soluble material subjected to silica SPE. Fractions eluted by 25−50% EtOAc/hexane were subjected to normal-phase HPLC (2.0 mL/min isocratic 40% EtOAc/hexane through a Phenomenex spherex 5μ 250 × 10 mm silica column) to give thiocyanatin A (**1**) as a colorless oil (18.2 mg, 0.10% specimen dry weight). The silica SPE fraction eluted with 5% EtOAc/hexane was also subjected to normal phase HPLC as described above using 15% EtOAc/hexane as eluent to give the inseparable mixture of thiocyanatin B (**2a**) and C (**2b**) as a colorless oil (20.3 mg, 0.12% specimen dry weight).

Thiocyanatin A (1): stable colorless oil; $[\alpha]_D$ 0° (*c* 1.0 in $CHCl_3$); IR ν_{max} (film) 3500, 2155 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.2−1.5 (m, methylene envelope), 1.85 (quin, J = 7.4 Hz, $H_2$2, $H_2$15), 2.95 (t, J = 7.4 Hz, $H_2$1, $H_2$16), 3.6 (m, H8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 25.5 (t, C6, C10), 28.7, 28.8 (t, C4, C13), 29.2, 29.3, 29.4 (t, C5, C11, C12), 29.8 (t, C2, C15), 33.9, 34.0 (t, C1, C16), 37.3, 37.4 (t, C7, C9), 71.8 (d, C8), 112.4 (s, S**C**N); ESI(+)MS *m*/*z* 379 (M + Na, 65), 374 (M + NH_4, 100), 357 (M + H, 85); EIMS *m*/*z* 200 (80), 186 (78); HRESI(+)MS *m*/*z* 357.2022 (M + H) (calcd for $C_{18}H_{33}S_2N_2O$ 357.2034).

Thiocyanatin B and C (2a/2b): stable colorless oil; UV (EtOH) λ_{max} 203 nm (ϵ 900); IR υ_{max} (film) 2155 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.25−1.40 (m, methylene envelope), 1.43

(br quin, $J = \sim 7.0$ Hz, $H_2$3, $H_2$14), 1.82 (quin, $J = 7.4$ Hz, $H_2$2, $H_2$15), 1.97 (m, $H_2$6 [2b], $H_2$7 [2a], $H_2$9 [2b], $H_2$10 [2a]), 2.95 (t, $J = 7.4$ Hz, $H_2$1, $H_2$16), 5.38 (m, H7 [2b], H8, H9 [2a]); ^{13}C NMR (100 MHz, $CDCl_3$) **2a** δ 27.9 (t, C3, C14), 28.7, 28.76, 29.36 (t, C4 and C13, C5 and C12, C6 and C11), 29.8 (t, C2, C15), 32.4 (t, C7, C10), 34.0 (t, C1, C16), 112.4 (s, S**C**N), 130.2 (d, C8, C9); **2b** δ 27.7, 27.9 (t, C3, C14), 28.3, 28.8, 28.9, 29.1, 29.2, 29.4 (t, C4, C5, C10, C11, C12, C13), 29.8 (t, C2, C15), 32.3, 32.5 (t, C6, C9), 34.0 (t, C1, C16), 112.4 (s, S**C**N), 129.9, 130.6 (d, C7, C8); ESI(+)MS *m/z* 361 (M + Na, 55), 356 (M + NH_4, 100), 339 (M + H, 40); HRESI(+)MS *m/z* 339.1919 (M + H) (calcd for $C_{18}H_{31}S_2N_2$ 339.1929).

(*Z*)-8-Hexadecenedioc Acid Dimethyl Ester (6). To a solution of **5** (6.00 g, 12 mmol) in dry THF (48 mL) and dry DMPU (16 mL) stirrred under N_2 at room temperature was added dropwise NaHMDS (12 mL, 12 mmol, 1 M solution in THF). The subsequent red solution was stirred at room temperature for 30 min, and then O_2 was bubbled into the reaction mixture. Stirring was continued at 60 °C for 16 h, after which time the red color of the reaction mixture had dissipated to a pale yellow. The reaction was quenched with saturated NH_4Cl(aq) (15 mL) and the mixture poured into water (150 mL). Standard workup with EtOAc, followed by column chromatography (silica gel, 10% EtOAc/hexane), afforded the diester **6** as a colorless oil (1.35 g, 72%):[22,23] IR υ_{max} (film) 1743 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$,) δ 1.2−1.3 (m, methylene envelope), 1.5−1.6 (m, $H_2$3, $H_2$14), 1.9−2.0 (m, $H_2$7, $H_2$10), 2.25 (t, $J = 7.5$ Hz, $H_2$2, $H_2$15), 3.60 (s, 2 × OMe), 5.3 (m, H8, H9); ^{13}C NMR (75 MHz, $CDCl_3$) 24.8, 27.0, 28.8, 28.9, 29.4, 33.9, 51.3, 129.7, 174.1; ESI(+)MS *m/z* 335 (M + Na, 100).

1,8,16-Trihydroxyhexadecane (7). To a solution of **6** (1.24 g, 3.96 mmol) in dry CH_2Cl_2 (120 mL) under N_2 at room temperature was added *m*-CPBA (1.30 g, 7.53 mmol). Stirring was continued for 16 h, then the reaction mixture was washed with saturated $NaHCO_3$(aq) (3 × 100 mL) and water (3 × 100 mL) and dried (anhyd Na_2SO_4) and the solvent removed under reduced pressure. Purification of the residue by column chromatography (silica gel, 35% EtOAc/hexane) gave the epoxide as a pale yellow oil (0.99 g, 76%), which was used in the next step without further purification. To a stirred suspension of $LiAlH_4$ (0.25 g, 6.6 mmol) in dry Et_2O (10 mL) under N_2 at room temperature was added dropwise a solution of the epoxide (0.43 g, 1.32 mmol) in dry Et_2O (10 mL) so as to maintain a gentle reflux. Refluxing was continued by heating for 20 h, and then the reaction was quenched with EtOAc followed by addition of 1 M HCl (20 mL) and water (20 mL). The aqueous phase was extracted with Et_2O (3 × 40 mL) and the combined organic extract washed with 1 M HCl (3 × 100 mL), water (3 × 100 mL), and brine (100 mL) and dried (anhyd $MgSO_4$). Removal of the solvent under reduced pressure gave the triol **7** (0.29 g, 80%) as a white solid that was recrystallized from hexane/EtOAc: mp 72−73 °C (lit.[20] mp 78−79.5 °C);. IR υ_{max} (KBr) 3308 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$,) δ 1.3−1.6 (m, methylene envelope), 3.59 (m, H8), 3.64 (t, $J = 6.6$ Hz, $H_2$1, $H_2$16); ESI(+)MS *m/z* 297 (M + Na, 100); HRESI(+)MS *m/z* 297.2403 (M + Na) (calcd for $C_{16}H_{34}O_3Na$ 297.2407).

Synthetic Thiocyanatin A ((±)-1). To a stirred suspension of the triol **7** (0.20 g, 0.74 mmol), DMAP (6.5 mg, 0.053 mmol), and TsCl (0.28 g, 1.47 mmol) in dry CH_2Cl_2 (5.5 mL) was added Et_3N (0.21 mL, 1.48 mmol) at 0 °C. After the mixture was stirred at room temperature for 16 h, additional TsCl (0.28 g, 1.47 mmol) and Et_3N (0.21 mL, 1.48 mmol) were added and stirring continued at room temperature for a further 16 h. The reaction mixture was concentrated under reduced pressure and the residue triturated with EtOAc. The EtOAc extract was concentrated under reduced pressure and subjected to silica column chromatography (20−40% EtOAc/hexane) to give the ditosylate **8** as a colorless oil (0.21 g, 49%), which was used in the next step without further purification. A mixture of **8** (95 mg, 0.16 mmol) and KSCN (40 mg, 0.41 mmol) in dry THF (5 mL) was refluxed under N_2 for 20 h. The reaction was cooled to room temperature and the THF removed under reduced pressure. Water (50 mL) was added, and then standard workup with Et_2O followed by silica column chromatography (30% EtOAc/hexane) yielded racemic thiocyanatin A ((±)-**1**) (45 mg, 77%) as a colorless oil that was spectroscopically identical to the natural thiocyanatin A (**1**).

Synthetic Thiocyanatin B and C (2a/2b). A mixture of the ditosylate **8** (100 mg, 0.17 mmol) and TsOH (10 mg, 0.053 mmol) in toluene (10 mL) was heated at reflux for 16 h. The toluene was removed under reduced pressure and the residue purified by silica column chromatography (10−20% EtOAc/hexane) to give the crude ditosyl alkene mixture **9** as an oily solid (45 mg, 46%), which was used in the next step without further purification. A mixture of ditosyl alkenes **9** (45 mg, 0.080 mmol) and KSCN (23.3 mg, 0.24 mmol) in dry THF (10 mL) was heated at reflux for 18 h. The reaction was cooled to room temperature and the solvent removed under reduced pressure. Water (50 mL) was added, and then standard workup with Et_2O afforded a mixture of dithiocyanate alkene regioisomers **2a/2b** as a clear oil (27.0 mg, 100%), which was spectroscopically identical to the natural thiocyanatin B and C mixture.

(*Z*)-1,16-Dihydroxy-8-hexadecene (10). To a mixture of $LiAlH_4$ (0.1 g, 2.6 mmol) in dry Et_2O (5 mL) under N_2 was added dropwise a solution of the diester alkene **6** (0.26 g, 0.84 mmol) in dry Et_2O (5 mL) at such a rate as to maintain a gentle reflux. Once the addition was complete, reflux was continued for 1 h. The reaction mixture was cooled, quenched with EtOAc, and 1 M HCl (10 mL) added followed by water (10 mL). The aqueous phase was extracted with Et_2O (3 × 20 mL), and the combined organic extract was washed with 1 M HCl (3 × 50 mL), water (3 × 50 mL), and brine (50 mL) and then dried (anhyd $MgSO_4$). The Et_2O was removed under reduced pressure to give the diol **10** as a colorless semisolid (0.18 g, 86%), which was recrystallized from hexane, EtOAc: mp 40−41 °C; IR υ_{max} 3400 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2−1.6 (m, methylene envelope), 1.9−2.1 (m, $H_2$7, $H_2$10), 3.64 (t, $J = 6.6$ Hz, $H_2$1, $H_2$16), 5.3−5.4 (m, H8, H9); ESI(+)MS *m/z* 279 (M + Na, 100); HRESI(+)MS *m/z* 295.2031 (M + K) (calcd for $C_{16}H_{32}O_2K$ 295.2041).

(*Z*)-1,16-Dithiocyanato-8-hexadecene (12). A mixture of diol **10** (0.18 g, 0.71 mmol) and Et_3N (0.25 mL, 1.78 mmol) in dry CH_2Cl_2 (8 mL) was stirred at −10 °C under N_2. MsCl (0.12 mL, 1.49 mmol) was added dropwise and stirring continued at −10 °C for 1 h, and then the mixture was poured onto ice and the organic phase separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic extract washed with ice-cold water (2 × 20 mL), 4% oxalic acid solution (20 mL), 5% $NaHCO_3$(aq) (20 mL), and brine (20 mL), and then dried (anhyd Na_2SO_4). The CH_2Cl_2 was removed under reduced pressure to give the dimesylate **11** as a colorless oil (0.28 g, 95%) that was used without further purification in the next step. A mixture of the dimesylate **11** (0.32 g, 0.78 mmol) and KSCN (0.19 g, 1.95 mmol) in EtOH (10 mL) was refluxed under N_2 for 16 h. The EtOH was removed under reduced pressure and water (20 mL) added to the residue. Standard workup (Et_2O) followed by silica column chromatography (5% EtOAc/hexane) afforded the dithiocyanate **12** as a colorless oil (0.16 g, 60%): IR υ_{max} (film) 2152 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.3−1.45 (m, methylene envelope), 1.80 (quin, $J = 7.4$ Hz, $H_2$2, $H_2$15), 1.9−2.1 (m, $H_2$7, $H_2$10), 2.92 (t, $J = 7.4$ Hz, $H_2$1, $H_2$16), 5.3−5.4 (m, H8, H9); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0 (C7, C10), 27.8 (C3, C14), 28.6, 28.8, 29.4 (C4 and C13, C5 and C12, C6 and C11), 29.7 (C2, C15), 33.9 (C1, C16), 112.3 (S**C**N), 129.7 (C8, C9); ESI(+)MS *m/z* 361 (M + Na, 100); HRESI(+)MS *m/z* 361.1744 (M + Na) (calcd for $C_{18}H_{30}S_2N_2Na$ 361.1751).

Acknowledgment. We acknowledge the CSIRO Division of Oceanography and the crew and scientific personnel aboard the O. R. V. Franklin for collection of the *Oceanapia* specimen. We also acknowledge technical support by A. Loveless, taxonomic classification by L. Goudie, and high-resolution mass measurements by S.

(22) Baker, R.; Crimmin, M. J. *Tetrahedron Lett.* **1977**, 441−442.
(23) Gensler, W. J.; Prasad, R. S.; Chaudhuri, A. P.; Alam, I. *J. Org. Chem.* **1979**, *44*, 3643−3650.

Duck of Monash University. This research was supported by the Australian Research Council and Novartis Animal Health Australasia Pty Ltd.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR data for the natural thiocyanatins (**1** and **2a/2b**) and the synthetic compound **12**, as well as IR, DEPT NMR, and MS data for natural **1** and **2a/2b**. COSY NMR data for natural **2a/2b**, ^{1}H NMR data for **6**, **7**, and **10**, and synthetic samples of thiocyanatins (**1** and **2a/2b**). This material is available free of charge via the Internet at http://pubs.acs.org.

JO0106750