

Nematocidal Thiocyanatins from a Southern Australian Marine Sponge *Oceanapia* sp.[†]

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

Centre for Molecular Biodiversity, Institute for Molecular Biosciences, University of Queensland, St. Lucia, Queensland 4072, Australia, School of Chemistry, University of Melbourne, Parkville, Victoria 3010, Australia, Microbial Screening Technologies Pty. Ltd., Kemp's Creek, New South Wales 2171, Australia, and Novartis Animal Health Pty. Ltd., Kemp's Creek, New South Wales 2171, Australia

Received January 6, 2004

Investigations of a southern Australian marine sponge, *Oceanapia* sp., have yielded two new β methyl branched bithiocyanates, thiocyanatins D₁ (**3a**) and D₂ (**3b**), along with two new thiocarbamate thiocyanates, thiocyanatins E₁ (**4a**) and E₂ (**4b**). The new thiocyanatins belong to a rare class of bioactive marine metabolite previously only represented by thiocyanatins A–C (**1**, **2a/b**). Structures were assigned on the basis of detailed spectroscopic analysis, with comparisons to the known bithiocyanate thiocyanatin A (**1**) and synthetic model compounds (**5**–**7**). The thiocyanatins exhibit potent nematocidal activity, and preliminary structure–activity relationship investigations have confirmed key characteristics of the thiocyanatin pharmacophore.

Recently we reported¹ the isolation, structure elucidation, synthesis, and biological activity of the first examples of a family of novel bithiocyanate nematocides, thiocyanatins A–C (**1**–**2a/b**), isolated from a southern Australian marine sponge, *Oceanapia* sp. Marine natural products possessing a thiocyanate functionality are rare, with the earliest reports (1989 and 1992) by Faulkner et al. describing sesquiterpene monothiocyanates from a Palauan sponge, *Axinyssa* (= *Trachyopsis*) *aplysinoidea*.^{2,3} By comparison, the thiocyanatins are acyclic lipids with twin terminal thiocyanate moieties. The closest known natural analogues to the thiocyanatins are also marine metabolites, being a selection of bisisothiocyanates reported (1987) by Scheuer et al. from a Fijian sponge, *Pseudaxinyssa* sp.⁴ Our ongoing interest in the thiocyanatins stems from the observation that thiocyanatin A (**1**) exhibits potent nematocidal activity against the commercial livestock parasite *Haemonchus contortus* (LD₉₉ = 1.3 μ g/mL). Furthermore, the thiocyanatins feature a structure motif readily accessible by synthesis. In light of these properties we were keen to further explore our *Oceanapia* sp. extract as a possible source of new bioactive thiocyanatins, to extend the selection of available natural thiocyanatins and further define the pharmacophore. In this report we describe four new nematocidal thiocyanatins as pairs of inseparable isomers; the isomeric β methyl branched bithiocyanates, thiocyanatins D₁ (**3a**) and D₂ (**3b**), and the isomeric thiocyanate-thiocarbamates, thiocyanatins E₁ (**4a**) and E₂ (**4b**).

Results and Discussion

As reported earlier,¹ the crude ethanolic extract from an *Oceanapia* sp. collected off southern Australia during scientific trawling operations exhibited significant nematocidal activity against the commercial livestock parasite

Haemonchus contortus (LD₉₉ = 135 μ g/mL). The extract was decanted, concentrated in vacuo, and triturated with CH₂Cl₂ to afford soluble material that displayed enhanced nematocidal activity. Further solvent triturations using hexane/diethyl ether mixtures followed by C₁₈ HPLC afforded the previously isolated metabolites thiocyanatins A–C (**1**–**2a/b**), as well as a series of new closely related analogues (**3a/b** and **4a/b**). The nematocidal activity was attributed to thiocyanatin A (LD₉₉ = 1.3 μ g/mL), as well as inseparable mixtures of thiocyanatins D₁ (**3a**) and D₂ (**3b**) (LD₉₉ = 3.1 μ g/mL) and thiocyanatins E₁ (**4a**) and E₂ (**4b**) (LD₉₉ = 8.3 μ g/mL).

High-resolution (+)ESIMS analysis of the inseparable mixture **3a/b** yielded a pseudomolecular ion ($M + Na$, m/z 393.1995) consistent with a molecular formula (C₁₉H₃₄S₂N₂O, Δ mmu = –3.9) requiring 4 DBE. A strong and characteristic IR absorbance (2156 cm^{–1}) was consistent with incorporation of all 4 DBE and the N and S heteroatoms into two thiocyanate functionalities, an observation further supported by the appearance of two nonequivalent thiocyanate ¹³C NMR resonances (¹³C, 112.3 (C) and 112.8 (C) ppm). The NMR data for **3a/b** (see Table 1) also revealed a 2°-OH methine (¹H, δ 3.58 (m); ¹³C, 71.9 (CH)), comparable to that observed in thiocyanatin A, as well as a 2°-Me (¹H, δ 1.05 (CH); ¹³C, 18.6 (CH₃)) β to a deshielded diastereotopic terminal methylene (¹H, δ 2.79 (J = 12.6, 7.5 Hz) and 3.00 (J = 12.6, 5.6 Hz); ¹³C, 41.4 (CH₂) ppm). Analysis of the 2D NMR COSY and gHMBC spectra for **3a/b** clearly supported the latter spin system (with correlations across the subunit –CH(CH₃)CH₂SCN), as did ¹H NMR comparisons with the synthetic model compound (*S*)-2-methylbutyl thiocyanate (**5**)⁶ (see Table 1). As with thiocyanatin A (**1**), NMR analysis of **3a/b** was unable to unambiguously position the 2°-OH on the carbon chain. Further to this, the ¹³C NMR spectrum of **3a/b** revealed a doubling of selected resonances that was interpreted as being due to 2°-OH regioisomers brought about by the unsymmetrical nature of the termini (branched vs unbranched).

As was the case with thiocyanatin A (**1**), placement of the 2°-OH along the alkyl chain in **3a/b** was achieved by interpretation of key fragmentations in the EI mass

[†] Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

^{*} To whom correspondence should be addressed. Tel: +61 7 334 62979. Fax: +61 7 334 62101. E-mail: r.capon@imb.uq.edu.au.

[†] University of Queensland.

[‡] University of Melbourne.

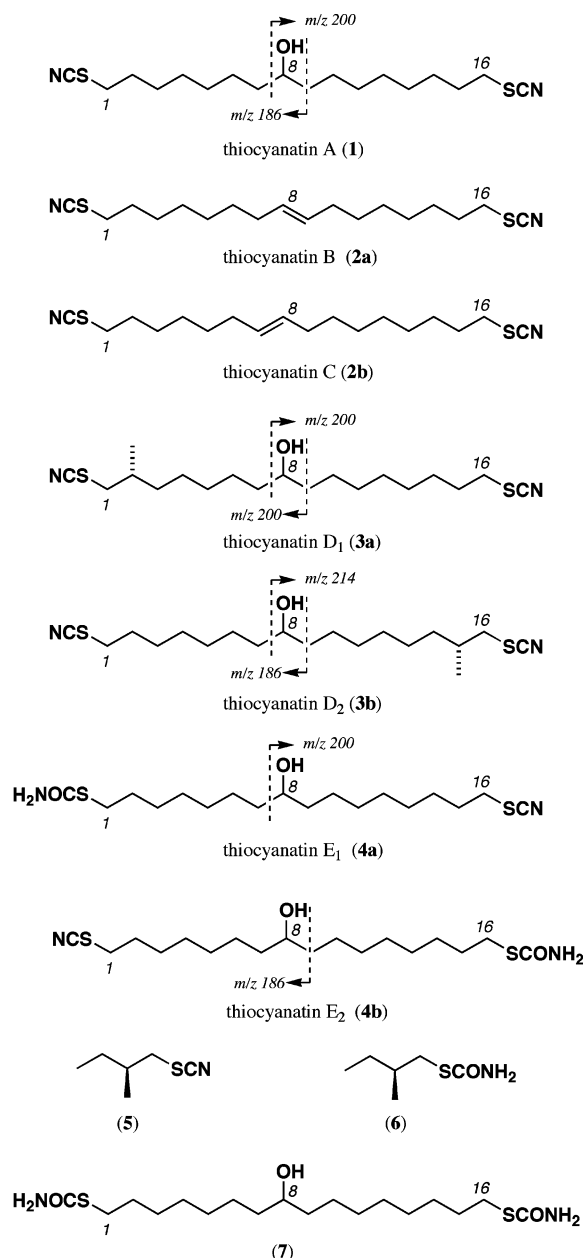
[§] Microbial Screening Technologies Pty. Ltd.

[#] Novartis Animal Health Pty. Ltd.

Table 1. ^1H NMR (400 MHz, CDCl_3) Data for Thiocyanatins A (**1**), $\text{D}_{1/2}$ (**3a/b**), and $\text{E}_{1/2}$ (**4a/b**) and the Models **5** and **7**

position	1	3a/b	4a/b ^a	5 ^b	7 ^a
H ₂ -1	2.95, t (7.4) ^l	2.79, dd (7.5, 12.6) ^c 3.00, dd (5.6, 12.6) ^c	2.81, t (7.2) ^h	2.80, dd (7.6, 12.9) 3.01, dd (5.7, 12.9)	2.81, t (7.2) ^l
H-2/H ₂ -2	1.85, m (7.4) ^m	1.9, m ^d 1.05, d (6.8) ^e	1.58, quin (7.1) ⁱ	1.82, m 1.05, d (6.6)	1.58, quin (7.2) ^m
CHOH	3.60, m	3.58, m	3.50, m		3.50, m
H ₂ -15	<i>n</i>	1.83, quin (7.4) ^f	1.81, quin (7.3) ^j		<i>p</i>
H ₂ -16	<i>o</i>	2.94, t (7.4) ^g	3.02, t (7.2) ^k		<i>q</i>
CH ₂ 's	1.2–1.5, m	1.2–1.7, m	1.2–1.5, m	1.2–1.6, m (H ₂ -3)	1.2–1.7, m

^a 400 MHz, d_4 -MeOH. ^b 300 MHz, CDCl_3 . ^c **3b** H₂-16. ^d **3b** H-15. ^e **3b** 15-Me. ^f **3b** H₂-2. ^g **3b** H₂-1. ^h **4b** H₂-16. ⁱ **4b** H₂-15. ^j **4b** H₂-2. ^k **4b** H₂-1. ^l Also H₂-16. ^m Also H₂-15. ⁿ As for H₂-2. ^o As for H₂-1.



spectrum. Diagnostic C–C cleavage α to the 2°-OH in **3a/b** yielded ions of the general formula $[\text{NCS}(\text{CH}_2)_n\text{HC}=\text{OH}]^{+\cdot}$ at m/z 186, 200, and 214. The proposed structure for thiocyanatin D₁ (**3a**) is as indicated since this is the only 2°-OH regioisomer capable of giving rise to an ion at m/z 200 (as shown in the structure diagram). Similarly, the favored structure for **3b** as shown represents an alternate biosynthetic alkylation of thiocyanatin A (**1**). In this way thiocyanatins D₁ (**3a**) and D₂ (**3b**) can be viewed as two alternate biosynthetic β methyl branched analogues of

thiocyanatin A (**1**), much the same as thiocyanatins B (**2a**) and C (**2b**) are alternate dehydration products of thiocyanatin A (**1**).

The inclusion of a β methyl branch in **3a/b** compared to thiocyanatin A (**1**) introduces a second asymmetric center. Whereas thiocyanatin A (**1**) possessed a single chiral center about the 2°-OH, the pseudo-symmetric nature of the molecule made it impossible to determine the enantio purity of **1**; thiocyanatin A (**1**) did not exhibit an optical rotation. It has been shown for a large number of labdane diterpenes in which the asymmetric ring system is separated by two methylene groups from an asymmetric center in the side chain that the molar rotation contributions for the two chiral subunits are additive.⁷ We have successfully used this technique to assign absolute stereochemistry to a number of marine natural products.^{8–14} Insofar as thiocyanatins D₁ (**3a**) and D₂ (**3b**) possess a “chiral” 2°-OH comparable to thiocyanatin A (**1**), it is reasonable to assume that this functionality does not contribute significantly to the optical properties of the **3a/b** mixture, requiring that the observed $[\Phi]_D = -13.7^\circ$ be largely if not solely due to the β methyl branched chiral center. Comparison of this measurement with that reported for the known (*S*)-2-methylbutyl thiocyanate (**5**)⁶ ($[\Phi]_D = +23.2^\circ$) supports assignment of an *R* stereochemistry about the β methyl branch in both thiocyanatins D₁ (**3a**) and D₂ (**3b**).

High-resolution (+)ESIMS analysis of the inseparable mixture **4a/b** yielded a pseudomolecular ion ($M + \text{Na}$, m/z 397.1949) consistent with a molecular formula $(\text{C}_{18}\text{H}_{34}\text{S}_2\text{N}_2\text{O}_2, \Delta \text{mmu} = -2.7)$ requiring 3 DBE and leading to the initial impression that **4a/b** were monohydrolysis analogues of thiocyanatin A (**1**). The NMR data for **4a/b** (see Table 1) revealed discrete resonances for the two terminal methylene groups, one substituted by a thiocyanate (^1H , δ 3.02 (m); ^{13}C , 34.9 (CH₂); gHMBC correlation $\text{CH}_2\text{--SCN}$ (113.7 ppm)) and the other substituted by a thiocarbamate (^1H , δ 2.81 (m); ^{13}C , 30.4 (t) ppm; gHMBC correlation $\text{CH}_2\text{--SCONH}_2$ (172.0 ppm)). These observations, together with doubling of selected resonances in the ^{13}C NMR spectrum, suggested that **4a/b** was a mixture of 2°-OH regioisomers of a mono-thiocarbamate analogue of thiocyanatin A (**1**). EI mass spectrometric analysis of **4a/b** revealed intense ions at m/z 186 and 200 of the general formula $[\text{NCS}(\text{CH}_2)_n\text{HC}=\text{OH}]^{+\cdot}$, consistent with the regioisomeric structures as shown, thiocyanatins E₁ (**4a**) and E₂ (**4b**). To confirm this assignment, the model bis-thiocarbamate **7** was prepared (63%) from methanolic HCl hydrolysis of synthetic thiocyanatin A (**1**). It is noteworthy that the NMR data for the thiocarbamate terminus in the model compound **7** were in excellent agreement with that recorded for **4a/b** (see Table 1), and the EI mass spectrum for **7** did not reveal diagnostic C–C cleavages α to the 2°-OH. This latter observation is consistent with the interpretation of the diagnostic ions in the EI mass spectrum of **4a/b** and may reflect the suscep-

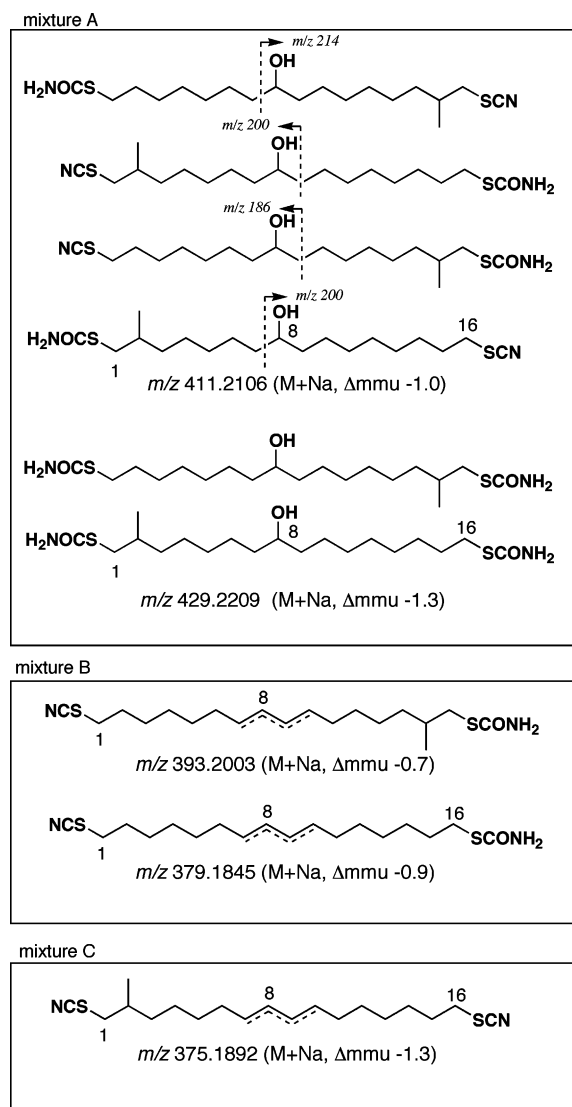


Figure 1. Minor thiocyanatins detected and *tentatively* identified in mixtures A–C.

tibility of the thiocarbamate functionality to EI fragmentation in advance of C–C cleavage α to the 2°-OH.

Our analysis of the *Oceanapia* extract also yielded three minor thiocyanatin fractions (mixture A (<0.5 mg), mixture B (<0.7 mg), and mixture C (<4 mg)) eluting as single peaks by C₁₈ HPLC.

¹H NMR and (+)ESI-HRMS analysis of mixture A suggested a selection of β methyl branched and further hydrolyzed (SCN to SCONH₂) analogues of thiocyanatins E₁/E₂ (**3a/b**). More specifically, resonances in the ¹H NMR (CDCl₃) spectrum for mixture A attributed to 2°-methyls (δ 1.05 and 0.95) and deshielded diastereotopic methylene protons (δ 2.68 and 2.78) were consistent with β methyl branching adjacent to both SCN and SCONH₂ termini. This analysis was confirmed by direct spectroscopic comparison with authentic samples of both the *S* thiocyanate **6** and its thiocarbamate analogue **7**, which revealed a characteristic ¹H NMR chemical shift difference between a 2°-Me β to a SCN ($\sim\delta$ 1.05) versus a SCONH₂ ($\sim\delta$ 0.95). The remaining ¹H NMR (CDCl₃) spectral data for mixture A were consistent with key resonances in thiocyanatins E₁/E₂ (**3a/b**), while the ¹³C NMR (CDCl₃) data revealed a resonance (20 ppm) consistent with the new β branched methyl termini. A list of plausible mixture A components is shown in Figure 1. Although an inseparable mixture by

C₁₈ HPLC, mixture A was resolved by GC into six components, four of which eluted early with similar retention times (42.3 (3%), 43.2 (18%), 43.6 (15%), and 43.8 (11%) min), while the remaining two eluted several minutes later, also with similar retention times (51.4 (28%) and 51.6 (24%) min). This GC analysis suggests that mixture A features most if not all the proposed structures shown in Figure 1.

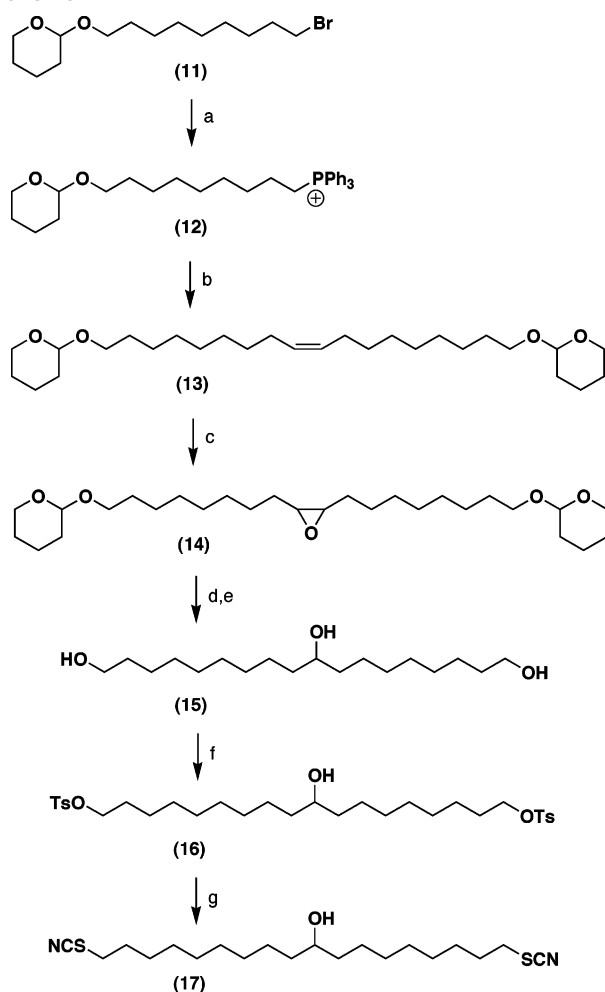
Analysis of the ^1H NMR and (+)ESI-HRMS data for mixture B suggested dehydration analogues along the lines of those indicated in Figure 1. The presence of only a single ^1H NMR (CDCl_3) 2°-Me resonance (δ 0.95) was consistent with β methyl branching adjacent to an SCONH_2 terminus, while the remaining ^1H NMR resonances compared well with thiocyanatins B and C (**2a/b**). On the basis of the hypothesis that the alkene thiocyanatins are biosynthetic dehydration products of the corresponding natural alcohols,¹ and given that the SCONH_2 functionality most likely arises from nonregiospecific hydrolysis of SCN moieties in suitable natural precursors, SCN and SCONH_2 asymmetrically terminated thiocyanatins would be expected to give rise to multiple alkene regioisomers (such as shown in Figure 1). GC analysis of mixture B revealed at least one major (95%) and one minor (5%) component (eluting at 50.7 and 51.0 min, respectively).

¹H NMR and (+)ESI-HRMS analyses of mixture C were consistent with β methyl branched analogues of thiocyanatins B and C (**2a/b**), which based on arguments comparable to those detailed above indicated that mixture C may be comprised of a selection of alkene regioisomers (see Figure 1). GC analysis of mixture C revealed major components eluting at 43.2 (38%) and 51.2 (45%) min, with additional minor components eluting at 42.2 (8%) and 50.8 (9%) min, not inconsistent with the proposed series of regio- and stereoisomeric alkenes shown in Figure 1.

While we acknowledge that the complex status of mixtures A–C precludes unambiguous structure assignments, the observations summarized above and in Figure 1 are disclosed at this time as an illustration of the breadth of metabolic variety that *Oceanapia* assembles around the thiocyanatin molecular motif.

In summary, the *Oceanapia* sp. has yielded a remarkable new class of natural products, several examples of which display potent nematocidal activity. In addition to isolating and identifying thiocyanatins A (**1**), B (**2a**), C (**2b**), D₁ (**3a**), D₂ (**3b**), E₁ (**4a**), and E₂ (**4b**) and drawing attention to a series of minor metabolites (Figure 1), we have prepared the synthetic β methyl branched thiocyanate **5** and thiocarbamate **6** and the bithiocarbamate **7**. We have also prepared monothiocyanate (**8**) and trithiocyanate (**9**) analogues of thiocyanatin A (**1**), as well as the deoxy analogue **10**. Furthermore, as outlined in Scheme 1, we have prepared a chain elongated C₁₈ homologue **17** of thiocyanatin A (**1**). Together with natural thiocyanatins, these synthetic analogues provide some preliminary SAR observations, based around the most potent natural example of this structure class, thiocyanatin A (**1**). Some noteworthy analogues and their nematocidal activity (LD₉₉) are listed in Figure 2. Even this limited selection of analogues highlights the importance of both the 2°-alcohol and SCN functionalities and the influence of chain length on nematocidal activity.

While the nematocidal mode of action of the thiocyanatins has yet to be determined, it is interesting to note that synthetic thiocyanates attracted early attention as potential insecticides. Researchers in the mid to late 1900s observed that alkyl thiocyanates could under appropriate enzymatic conditions release HCN, and they went on to

Scheme 1^a

^a a, PPh₃; b, NaHMDS, O₂; c, mCPBA; d, LAH; e, H⁺; f, TsCl; g, KSCN.

explore the agrochemical potential of this effect. For example, C₁₂ and C₁₃ *n*-alkyl and *sec*-alkyl thiocyanates were noted to be outstanding insecticides against adult female fruit-tree red spider mites,¹⁵ while various other alkyl thiocyanates were described as lethal to mice and houseflies¹⁶ and selected beetles¹⁷ through the action of HCN. Although these early investigations focused on synthetic alkyl thiocyanates, it has long been known that certain plants and insects employ HCN as a defensive agent through the intermediary of cyanogenic glycosides. At this stage the chemical ecology of the thiocyanatins remains unknown, although it is tempting to speculate that naturally occurring alkyl thiocyanates such as the thiocyanatins may function as chemical defense agents, countering predatory attack through localized enzyme-mediated release of HCN.

Experimental Section

General Experimental Procedures. See ref 1.

Animal Material. See ref 1.

Extraction and Isolation. The EtOH extract of the *Oceanapia* sp. was decanted and concentrated in vacuo (800 mg), and the nematocidal components were extracted by trituration with CH₂Cl₂ (329 mg), as detailed in an earlier report.¹ The CH₂Cl₂-soluble material was then subjected to a further series of triturations to give hexane (92.6 mg), 1:1 hexane/Et₂O (103.1 mg), and Et₂O soluble (73.5 mg) along with an insoluble (54.3 mg) fraction. The hexane-soluble material was treated with MeOH (0.5 mL), the precipitated fats and steroids (12.4 mg) were removed, and the supernatant was

subjected to C₁₈ HPLC (2.0 mL/min 85–100% MeOH/water through a Zorbax Eclipse XDB 5 μ m 250 \times 9.4 mm C₁₈ column) to give in order of elution thiocyanatin A (1) (11.0 mg, 0.03%), an inseparable mixture of thiocyanatins D₁ and D₂ (3a/b) (4.6 mg, 0.013%), and an inseparable mixture of thiocyanatins B and C (2a,b) (23.2 mg, 0.066%). The 1:1 hexane/Et₂O-soluble material was treated in a similar fashion to give in order of elution an inseparable mixture of thiocyanatins E₁ and E₂ (4a/b) (5.4 mg, 0.015%), thiocyanatin A (1) (15.3 mg, 0.043%), an inseparable mixture of thiocyanatins D₁ and D₂ (3a/b) (3.8 mg, 0.011%), and an inseparable mixture of thiocyanatins B and C (2a,b) (1.6 mg, 0.004%). Also recovered were the HPLC inseparable mixtures A (<0.5 mg, <0.001%), B (<0.7 mg, <0.001%), and C (<4 mg, <0.01%). All reported yields are measured against the dry weight of the sponge sample. The known thiocyanatins A–C were identical in all respects to those previously reported.¹

Thiocyanatins D₁ and D₂ (3a/b): clear viscous oil; [α]_D²¹ –3.7° (c 0.41, CHCl₃); IR (CHCl₃) ν_{\max} 3500 (br), 2156 cm^{–1}; ¹H NMR (see Table 1); ¹³C NMR (100 MHz, CDCl₃) δ 18.6 (2-Me), 25.5, 25.6, 26.6, 27.86/27.91 (C-14), 28.78/28.82 (C-13), 29.3, 29.4, 29.5, 29.6, 29.9 (C-15), 33.8 (C-2), 34.1 (C-16), 35.2 (C-3), 37.4/37.5 (C-7/9 [3a], C-8/10 [3b]), 41.4 (C-1), 71.9 (C-8 [3a], C-9 [3b]), 112.3 (16-SCN), 112.8 (1-SCN); (+)ESIMS *m/z* 393 [(M + Na)⁺], 353 [(M + H)⁺ – H₂O]; (+)ESI-HRMS *m/z* 393.1995 [(M + Na)⁺, calcd for C₁₉H₃₄S₂N₂O₂Na, 393.2010].

Thiocyanatins E₁ and E₂ (4a/b): clear viscous oil; ¹H NMR (see Table 1); ¹³C NMR (100 MHz, d₄-MeOH) δ 26.6, 26.7, 28.9 (C-14), 29.7 (C-3), 29.9, 30.0, 30.2, 30.3, 30.4 (C-1), 30.5, 30.6, 30.69, 30.74, 31.1 (C-15), 31.6 (C-2), 34.9 (C-16), 38.4 (C-7/9 [4a], C-8/10 [4b]), 72.4 (C-8 [4a], C-9 [4b]), 113.7 (SCN), 172.0 (SCONH₂); (+)ESIMS *m/z* 397 [(M + Na)⁺]; (+)ESI-HRMS *m/z* 397.1949 [(M + Na)⁺, calcd for C₁₈H₃₄S₂N₂O₂Na, 397.1960].

(S)-2-Methylbutyl Thiocyanate (5).⁶ A mixture of LiAlH₄ (0.35 g, 9.18 mmol) and dry ether (15 mL) was added dropwise to a solution of (+)-(*S*)-2-methylbutyric acid (0.47 g, 4.59 mmol) in dry ether (10 mL) under gentle reflux. Stirring was continued at 40 °C for 16 h, after which the excess LiAlH₄ was filtered and the filtrate treated with EtOAc (20 mL) followed by 10% HCl_{aq} (20 mL). The aqueous phase was extracted with ether (10 mL \times 2) and the combined organic phase washed sequentially with 10% HCl_{aq} (10 mL), H₂O (10 mL), and brine (10 mL), then dried (anhydrous MgSO₄). The ether was removed under reduced pressure to give (*S*)-2-methylbutanol (0.17 g, 42%) as a pale yellow oil. The crude alcohol (0.17 g, 1.93 mmol) in dry CH₂Cl₂ (20 mL) was treated with Et₃N (0.4 mL, 2.90 mmol) and the mixture chilled to –10 °C, at which point methanesulfonyl chloride (0.15 mL, 2.04 mmol) was added dropwise and the reaction stirred at –10 °C for 1 h. The reaction mixture was poured onto ice and extracted with additional CH₂Cl₂ (10 mL \times 2), after which the combined organic extract was washed sequentially with ice-cold water (10 mL \times 2), 4% w/v aqueous oxalic acid (10 mL \times 2), 2% NaHCO₃ (10 mL \times 2), and brine (10 mL), then dried (anhydrous Na₂SO₄). The CH₂Cl₂ was removed under reduced pressure to give the (*S*)-2-methylbutyl methanesulfonate (0.16 g, 51%). A mixture of crude mesylate (0.16 mg, 0.96 mmol) and KSCN (93 mg, 0.96 mmol) in dry THF (20 mL) was stirred at reflux for 16 h. The reaction was cooled to RT and the solvent removed under reduced pressure. The residue was partitioned between H₂O (10 mL) and ether (10 mL) and further extracted with ether (10 mL \times 2), after which the combined organic phase was sequentially washed with H₂O (10 mL) and brine (10 mL) and then dried (anhydrous MgSO₄). Evaporation of the ether followed by elution through a silica SPE cartridge (eluent 8% EtOAc/hexane) yielded the desired (*S*)-2-methylbutyl thiocyanate (5) (20 mg, 16%) as a pale yellow oil, along with unreacted mesylate (57 mg): ¹H NMR (see Table 1); [α]_D¹⁴ +18.8° (c 1.0, CHCl₃); IR (film) ν_{\max} 2156 cm^{–1}; (+)ESIMS *m/z* 152 [(M + Na)⁺].

(S)-2-Methylbutyl Thiocarbamate (6). (*S*)-2-Methylbutyl thiocyanate (5) (8.4 mg, 0.065 mmol) was dissolved in MeOH (40 mL) and dry HCl gas passed through the solution for 2 h at 0 °C. The solution was then stirred at RT for 16 h before

was added. The mixture was stirred at reflux for 16 h, after which the solvent was removed under reduced pressure. The residue was partitioned between H₂O (10 mL) and Et₂O (10 mL), and the aqueous phase extracted with Et₂O (3 × 10 mL). The combined organic phase was washed with brine (10 mL), dried (anhydrous MgSO₄), filtered, and concentrated under reduced pressure. Purification by silica SPE cartridge using 5% EtOAc/hexane as eluent afforded the dithiocyanate **10** (0.13 g, 83%): IR ν_{\max} (film) 2152 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.23–1.29 (m, methylene envelope), 1.43 (m, 3-H₂, 14-H₂), 1.82 (quin, J = 7.5 Hz, 2-H₂, 15-H₂), 2.94 (t, J = 7.2 Hz, 1-H₂, 16-H₂); ¹³C NMR (100 MHz, CDCl₃) 27.9, 28.8, 29.3, 29.4, 29.5, 29.6, 29.8, 34.0, 112.4; (+)ESIMS m/z 363 (M + Na, 100), 341 (M + H, 60), 282 (M – SCN, 70); (+)ESI-HRMS m/z 363.1902 (M + Na) (calcd for C₁₈H₃₂N₂S₂Na 363.1905).

1,9,18-Trihydroxyoctadecane (15). A mixture of commercially available 9-bromo-1-nonanol (5 g, 22.4 mmol), dihydropyran (2.1 mL, 23.3 mmol), and concentrated HCl (22 μ L) was stirred at RT for 18 h. The reaction mixture was diluted with Et₂O (20 mL) and washed with H₂O (20 mL), saturated NaHCO_{3aq} (2 × 20 mL), and brine (20 mL), then dried (anhydrous MgSO₄), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 10% EtOAc/hexane as eluent yielded the THP-protected alcohol **11** (5.74 g, 84%). A mixture of **11** (5.74 g, 18.9 mmol) and PPh₃ (4.46 g, 18.9 mmol) in MeCN (45 mL) was stirred at reflux for 16 h, then concentrated under reduced pressure to give the corresponding Wittig salt **12** (10.6 g, 99%) as a colorless oil. To a stirred solution of **12** (4.89 g, 8.66 mmol) in dry THF (35 mL) and DMPU (11 mL) under N₂ at RT was added dropwise NaHMDS (8.66 mL, 8.66 mmol, 1 M solution in THF). Stirring was continued for 30 min, after which O₂ was passed into the reaction, and stirring continued at 55 °C for 16 h. Saturated NH₄Cl_{aq} was added and the mixture poured into H₂O (150 mL). The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phase washed with H₂O (2 × 100 mL), brine (100 mL), then dried (anhydrous MgSO₄), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 5% EtOAc/hexane as eluent yielded the dimer alkene **13** (1.11 g, 61%) as a clear oil: ¹H NMR (300 MHz, CDCl₃) δ 1.29 (m, methylene envelope), 1.49–1.63 (m, 3'-H₂, 4'-H₂, 8'-H₂, 9'-H₂), 1.72 (m, 2'-H₂, 7'-H₂), 1.82 (m, 7-H₂, 12-H₂), 2.01 (8-H₂, 11-H₂), 3.39 (dt, J = 9.6, 6.6 Hz, 1-H₂, 16-H₂), 3.50 (m, 5'-H₂, 10'-H₂), 3.72 (dt, J = 9.6, 6.9 Hz, 1-H₂, 16-H₂), 3.87 (m, 5'-H₂, 10'-H₂), 4.57 (t, J = 4.5 Hz, 1'-H₂, 6'-H₂), 5.34 (m, 9-H₂, 10-H₂); ¹³C NMR (75 MHz, CDCl₃) 19.6, 25.5, 26.2, 27.2, 29.2, 29.4, 29.5, 29.7, 30.7, 62.3, 67.6, 98.8, 129.8; (+)ESIMS m/z 285 (M – (THP)₂ + H, 100), 475 (M + Na, 50); (+)ESI-HRMS m/z 475.3746 (M + Na) (calcd for C₂₈H₅₂O₄Na 475.3763). A mixture of **13** (0.41 g, 0.91 mmol) and mCPBA (1.00 g, 5.3 mmol) in dry CH₂Cl₂ (80 mL) was stirred at RT under N₂ for 16 h. The reaction mixture was washed with saturated NaHCO_{3aq} (3 × 80 mL), H₂O (80 mL), and brine (80 mL) and dried (anhydrous MgSO₄), then filtered and concentrated under reduced pressure. Purification by silica column chromatography using 15% EtOAc/hexane as eluent afforded the corresponding epoxide **14** (0.31 g, 73%). To a stirred suspension of LiAlH₄ (0.13 g, 3.32 mmol) in dry Et₂O (20 mL) under N₂ at RT was added dropwise a solution of **14** (0.31 g, 0.66 mmol) in dry Et₂O (20 mL) maintaining a gentle reflux. Refluxing was continued for 18 h, and then the reaction was quenched with EtOAc followed by addition of 1 M HCl_{aq} (40 mL) and H₂O (40 mL). The aqueous phase was extracted with Et₂O (3 × 40 mL) and the combined organic extract washed with 1 M HCl_{aq} (3 × 40 mL), H₂O (3 × 40 mL), and brine (40 mL), then dried (anhydrous MgSO₄) and filtered. Removal of the solvent under reduced pressure yielded a product (0.30 g, 0.63 mmol), which was taken up in MeOH (2 mL) and treated with added concentrated H₂SO₄ (0.1 mL) at RT for 16 h, then concentrated under reduced pressure. The residue was diluted with Et₂O (25 mL) and washed with

saturated NaHCO_{3aq} (25 mL), H₂O (25 mL), and brine (25 mL), then dried (anhydrous Na₂SO₄). The crude material was recrystallized from hexane/EtOAc to yield 1,9,18-trihydroxyoctadecane (**15**) (61 mg, 32%) as a white solid: mp 58–62 °C; IR ν_{\max} (KBr) 3310 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.12–1.31 (m, methylene envelope), 1.36–1.45 (m, 2-H₂, 17-H₂), 3.42 (m, 9-H₂), 3.45 (t, J = 6.6 Hz, 1-H₂, 18-H₂); ¹³C NMR (100 MHz, CDCl₃) 26.6, 26.7, 26.8, 26.9, 30.3, 30.4, 30.5, 30.6, 30.7, 30.8, 38.5, 64.1, 73.1; (+)ESIMS m/z 325 (M + Na, 100), 341 (M + K, 10); (+)ESI-HRMS m/z 325.2710 (M + Na) (calcd for C₁₈H₃₈O₃Na 325.2719).

9-Hydroxy-1,18-dithiocyanato-octadecane (17). To a stirred suspension of **15** (61 mg, 0.20 mmol), DMAP (2.5 mg, 0.02 mmol), and *p*-TsCl (80.9 mg, 0.42 mmol) in dry CH₂Cl₂ (2 mL) was added Et₃N (59 μ L, 0.42 mmol) at 0 °C, and the mixture stirred at RT for 16 h, after which DMAP (1 mg, 8 μ mol), *p*-TsCl (40 mg, 0.21 mmol), and Et₃N (30 μ L, 0.21 mmol) was added at 0 °C. The reaction was stirred at RT for a further 16 h, then concentrated under reduced pressure. The residue was purified by silica chromatography using 30% EtOAc/hexane as eluent to yield 1,16-ditosylate **16** (52 mg, 42%). A mixture of **16** (52 mg, 0.085 mmol) and KSCN (21 mg, 0.21 mmol) in dry THF (10 mL) was stirred at reflux for 20 h, after which solvent was removed under reduced pressure and the residue partitioned between H₂O (10 mL) and Et₂O (10 mL). The aqueous phase was extracted with Et₂O (3 × 10 mL) and the combined organic phase washed with brine (10 mL), dried (anhydrous MgSO₄), filtered, and concentrated under reduced pressure. Purification by silica column chromatography using 20% EtOAc/hexane yielded 9-hydroxy-1,18-dithiocyanato-octadecane (**17**) (20 mg, 62%): IR ν_{\max} (KBr) 3503, 2152 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.29–1.52 (m, methylene envelope), 1.82 (quin, J = 7.8 Hz, 2-H₂, 15-H₂), 2.94 (t, J = 6.9 Hz, 1-H₂, 16-H₂), 3.58 (m, 9-H); ¹³C NMR (100 MHz, CDCl₃) 25.5, 25.6, 27.8, 27.9, 28.7, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8, 29.9, 34.0, 34.1, 37.4, 37.5, 71.9, 112.4; (+)ESIMS m/z 407 (M + Na, 100), 423 (M + K, 80); (+)ESI-HRMS m/z 407.2167 (M + Na) (calcd for C₂₀H₃₆N₂OS₂Na 407.2167).

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

References and Notes

- Capon, R. J.; Skene, C.; Liu, E.; Lacey, E.; Gill, J. H.; Heiland, K.; Friedel, T. *J. Org. Chem.* **2001**, *66*, 7765–7769.
- Yin, H. H.; Faulkner, D. J.; Shumsky, J. S.; Hong, K.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 2511–2514.
- Yin, H. H.; Salva, J.; Catalos, R. F.; Faulkner, D. J. *J. Org. Chem.* **1992**, *57*, 3191–3194.
- Karusu, P.; Scheuer, P. J. *Tetrahedron Lett.* **1987**, *28*, 4633–4636.
- Cheer, C. J.; Pickles, F. J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1805.
- Casey, J. P.; Martin, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 6141–6151.
- Carmen, R. M. *Aust. J. Chem.* **1966**, *19*, 629–642.
- Capon, R. J.; MacLeod, J. K. *J. Nat. Prod.* **1987**, *50*, 225–229.
- Capon, R. J.; Ghisalberti, E. L.; Jefferies, P. R. *Aust. J. Chem.* **1981**, *34*, 1775–1778.
- Capon, R. J.; Ghisalberti, E. L.; Jefferies, P. R. *Phytochemistry* **1983**, *22*, 1465–1467.
- Capon, R. J.; Faulkner, D. J. *J. Org. Chem.* **1984**, *106*, 1819–1822.
- Butler, M. S.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 77–85.
- Butler, M. S.; Capon, R. J. *Aust. J. Chem.* **1992**, *45*, 1705–1743.
- Capon, R. J.; Dargaville, T. R.; Davis, R. *Nat. Prod. Lett.* **1994**, *4*, 51–56.
- Eaton, J. K.; Davies, R. G. *Ann. Appl. Biol.* **1950**, *37*, 471–489.
- Ohkawa, H.; Ohkawa, R.; Yamamoto, I.; Casida, J. E. *Pesticide Biochem. Physiol.* **1972**, *2*, 95–112.
- Zsolnai, T. *Acta Phytopathol. Acad. Sci. Hung.* **1974**, *9*, 125–141.

NP049977Y