Linear and Cyclic C 18 Terpenoids from the Southern Australian Marine Brown Alga Cystophora moniliformis

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · SEPTEMBER 2008

Impact Factor: 3.8 · DOI: 10.1021/np800201c · Source: PubMed

2 AUTHORS:

Priyanka Reddy
Department of Environment and Primary I…
2 PUBLICATIONS **49** CITATIONS

SEE PROFILE

Sylvia Urban
RMIT University
77 PUBLICATIONS **829** CITATIONS

SEE PROFILE

Linear and Cyclic C_{18} Terpenoids from the Southern Australian Marine Brown Alga *Cystophora moniliformis*

Priyanka Reddy and Sylvia Urban*

School of Applied Sciences (Discipline of Applied Chemistry), RMIT University, GPO Box 2476V Melbourne, Victoria 3001, Australia

Received April 1, 2008

Chemical investigation of the Southern Australian marine brown alga *Cystophora moniliformis* resulted in the isolation of two new cyclic epimeric terpene diols, moniliforminol A (**25**) and moniliforminol B (**26**), a new linear farnesylacetone derivative (**27**), and the previously described terpenoids **19**−**24**. This study also resulted in the first complete 2D NMR characterization for compounds **21** to **24** as well as the first report of **24** as a natural product. All structures were elucidated by detailed spectroscopic analysis, with the relative configurations of **25** and **26** being established by selective 1D NOE NMR experiments. The absolute configuration of **26** was assigned on the basis of circular dichroism (CD), which also permitted that of **25** to be tentatively assigned.

There are an estimated 23 known *Cystophora* species, for which the phytochemistry of approximately 14 have been reported.[1,2] As a result of suitable environmental conditions that have enabled its evolution, *Cystophora* spp. are endemic to the cool temperate waters of Australasia.[2–4]

The two major genera of the Cystoseiraceae family include *Cystoseira* and *Cystophora*, and on the basis of existing phytochemical data the *Cystoseira* species were classified into three categories (A−C) based on the metabolites produced.[5] Category A comprises those species that do not contain diterpenes, category B includes species that produce linear diterpenes, and those in category C are species that contain meroditerpenoids.[5] The meroditerpenes have been further subdivided into three classifications, which include linear, cyclic, and rearranged terpenoids.[5] It was recognized that by identifying the degree of complexity in the terpenes produced by *Cystoseira* species, together with the morphological and reproductive data, differences in the level of phylogenetic advancement could be ascertained.[1,2,5,6] The assertion made was that terpenes of greater complexity (e.g., cyclic and rearranged meroditerpenoids) are phylogenetically more advanced than others.[5,6] The second major genus of the Cystoseiraceae family, *Cystophora*, as well as other genera within the Cystoseiraceae family, are known to produce both isoprenoid and nonisoprenoid secondary metabolites with various functionalized carbon skeletons, making it frequently difficult to classify these genera to the species level.[5] In many cases they are characterized by the presence of linear and cyclic C_{18} terpenoid metabolites such as compounds **1** to **9**, which can be regarded as being specific markers of the Australian genera.[5]

Generally the *Cystophora* spp. produce secondary metabolites such as phloroglucinols, halogenated phlorethols, polyenes, simple terpenes, and rarely meroditerpenoids.[6–21] The presence of terpenes as compared to acetogenins in brown algae has been suggested to be indicative of advanced secondary metabolism.[2] In the case of *Cystophora moniliformis,* a range of fairly simple farnesylacetone derivatives are known to be produced as well as simple meroditerpenoids.[6] The sole species producing metabolites other than only acetogenins is *C. moniliformis*, which has yielded variously functionalized and cyclized farnesylacetone derivatives such as compounds **1** to **12** as well as related metabolites including the tricyclic terpene **13** and the lactone **14**.[2] Wells and co-workers have reported the isolation of meroditerpenoids from *Cystophora moniliformis* including the chromanes, δ-tocotrienol (**15**), and α-tocopherol (**16**).[15] This further supports the proposed phylogeny that has classified *C. moniliformis* as being one of the most developed species of this genus based on morphology alone, as indicated by Womersley.[1,2] Phycologists have often found it difficult to taxonomically identify brown algae to the species level.[5] Additional information such as phytochemistry is useful for this systematic taxononomic classification, and it is here that chemotaxonomy can serve an important role.[2,5]

As part of the activities of the Marine and Terrestrial Natural Product (MATNAP) research group at RMIT University, which studies the chemistry and biological activity of southern Australian marine and terrestrial organisms, we examined a brown alga, *Cystophora moniliformis*, collected from Port Phillip Bay, Victoria, Australia. The chemotaxonomic profiling of the brown alga *Cystophora moniliformis* was stimulated on the basis of the moderate antitumor, antiviral, and antifungal activities displayed by the crude extract of this specimen. We describe here the isolation and structure determination of two new cyclic farnesylacetone terpenoids (**25** and **26**), a new linear terpenoid (**27**), and a mixture of the known compounds **19** and **20** together with the first complete 2D NMR characterization for the previously reported compounds **21**−**24**.

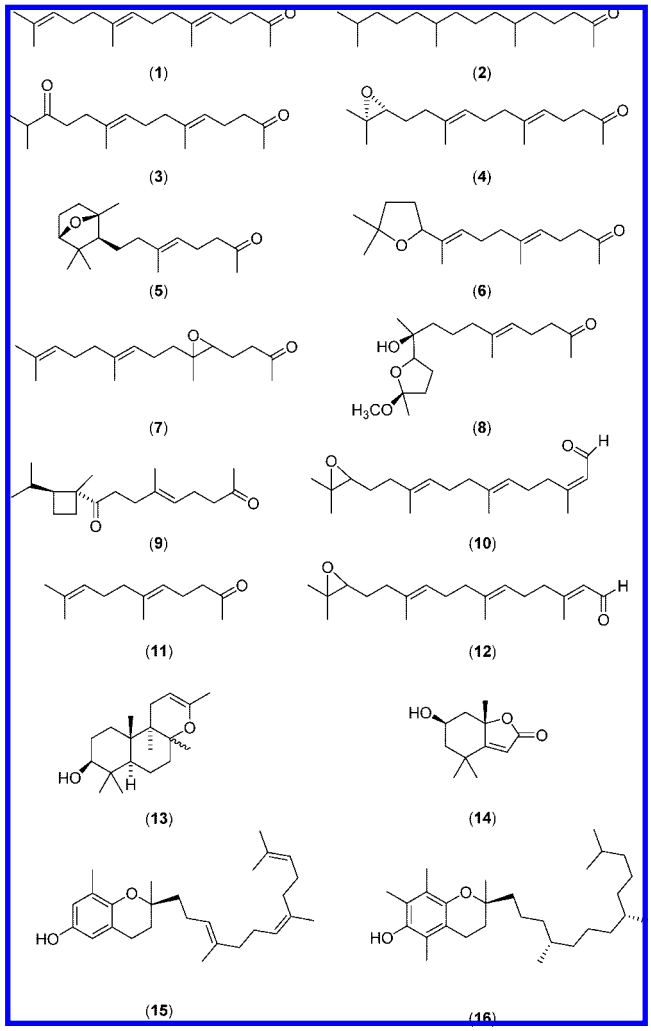
Results and Discussion

The alga was extracted with 3:1 CH_3OH/CH_2Cl_2 and then sequentially solvent partitioned with CH_2Cl_2 followed by CH_3OH. The CH_2Cl_2 partition was fractionated by silica gel flash chromatography, and the 40:60 CH_2Cl_2/EtOAc as well as the 80:20 CH_2Cl_2/EtOAc fractions were individually subjected to repeated gel permeation chromatography (Sephadex LH-20) using CH_3OH. Final purification was achieved by reversed-phase HPLC to yield terpenoids **19**−**27**.

Compounds **19** and **20** were isolated in this study as a mixture in a ratio of 3:1, respectively, and identified on the basis of a ^{1}H NMR comparison to the literature data.[15] As a result of their reisolation, additional chemical shift assignments for **19** and **20** could be made. Terpenoids **21**−**24** have also been previously reported but were identified solely on the basis of limited ^{1}H and ^{13}C NMR assignments and mass spectrometry.[15,22] As a result of this study, structures **21**−**24** were confirmed and fully assigned by detailed spectroscopic analysis including the first complete unequivocal assignment by 2D NMR spectroscopy. The absolute configuration for the secondary alcohol functionality in compounds **21** and **22** had been previously established by the Horeau method, while the acid-catalyzed rearrangement of compound **19** resulted in a mixture of compounds **19**, **20**, and **23**, which subsequently established the absolute configuration of these compounds.[15]

* Corresponding author. Tel: +61 3 9925 3376. Fax: +61 3 9925 3747. E-mail: sylvia.urban@rmit.edu.au.

10.1021/np800201c CCC: $40.75
Published on Web 08/08/2008



Moniliforminol A (**25**) was isolated as colorless oil for which HRESIMS established the molecular formula as $C_{18}H_{32}O_3$ (319.2252 $[M + Na]^+$, calcd for $C_{18}H_{32}O_3Na$, 319.2249) possessing three degrees of unsaturation. The IR spectrum supported the presence of hydroxy groups (3401 cm^{-1}), a ketone (1709 cm^{-1}), and an olefinic moiety (1589 cm^{-1}). Analysis of the NMR spectra (Table 1) revealed chemical shifts indicative of a methyl ketone (δ_H 2.13, δ_C 209.1), one olefinic methyl (δ_H 1.64, δ_C 16.3), three methyl singlets [(δ_H 0.79, δ_C 15.1), (δ_H 1.02, δ_C 28.3), (δ_H 1.15, δ_C 23.2)], one olefinic double bond [(δ_H 5.08, δ_C 123.1) and (δ_C 137.4, s)], and a secondary alcohol methine (δ_H 3.32, δ_C 78.4). DEPT and HSQCAD NMR experiments supported the presence of five methyl, six methylene, and three methine carbons, while the remaining quaternary carbons were identified from the HMBC experiment (Table 1). Both COSY and HMBC correlations as well as comparison to the literature NMR data for compound **21** readily established the presence of the linear terpene side-chain in the substructure of moniliforminol A (**25**).[15] On the basis of the molecular formula and IR and NMR data, one degree of unsaturation still needed to be accounted for. A combination of HMBC and COSY correlations (Table 1) established the presence of a six-membered ring, which accounted for the remaining degree of unsaturation. Linking of the linear terpene moiety to the six-membered ring was achieved through the observation of HMBC correlations from the methylenes at positions 7 and 8 to a methine at position 1′ on the six-membered ring. In moniliforminol A (**25**) the C-2′ and C-5′ carbons overlapped at δ_C 41.1.

Moniliforminol B (**26**) was immediately recognized to be an epimer of moniliforminol A (**25**) on the basis of the similarity of the IR and ^1H and ^{13}C NMR spectra (Table 2) between these two compounds. Moniliforminol B (**26**) was isolated as a colorless oil, and HRESIMS confirmed that **26** had the same molecular formula as **25**, $C_{18}H_{32}O_3$ (319.2251 $[M + Na]^+$, calcd for $C_{18}H_{32}O_3Na$, 319.2249). The IR spectrum of **26** was very similar to **25** and again supported the presence of the hydroxy moieties (3369 cm^{-1}), a ketone (1713 cm^{-1}), and an olefinic double bond (1589 cm^{-1}). Finally, the UV spectra and extinction coefficients were almost identical for both moniliforminols A (**25**) and B (**26**). Stereochemical assignment of the double bonds of terpenoids **19** to **27** was made on the basis of the position of the vinyl methyl resonances in the ^{13}C NMR spectrum (δ_C 15.9−16.4) for these compounds.[23,24] While the NMR data were very similar to **25**, all of the carbon signals were resolved in **26** (Table 2) including C-2′ and C-5′. The deshielded resonance attributed to C-7′ in **26** (δ_C 30.7) relative to **25** (δ_C 23.2) was interpreted as having a *cis* disposition with respect to H-1′ in **26**, while a *trans* disposition was concluded between C-7′ and H-1′ in **25**.[25] This conclusion is based on energy-minimized structures of **25** and **26**, which indicate additional interactions in **25** and provided further support for the epimeric disposition at position 6′.

The relative configuration of moniliforminol A (**25**) and moniliforminol B (**26**) was determined by single irradiation NOE experiments with selective irradiations shown in Figure 1. These key NOE enhancements confirmed that moniliforminol A (**25**) and moniliforminol B (**26**) were epimeric with a reversed orientation of the hydroxy and methyl substituents at C-6′. The ^1H NMR coupling constants of the hydroxy methine proton support the stereochemical assignment as equatorial in both **25** and **26**. After examination of the available literature[26–31] describing Cotton effects displayed by similar terpenoids, we were able to assign the absolute configuration to **26** on the basis of a positive Cotton effect ($\Delta\varepsilon_{230\ nm}$ +6.67) in the CD spectrum of this compound, which was compared with that observed for **28** ($\Delta\varepsilon_{239.5\ nm}$ +11.01).[26] The absolute configuration at C-3′ in **28** had been previously established by application of the Mosher method, which subsequently allowed the complete absolute configuration of **28** to be assigned.[26] The absolute configuration of **28** was further corroborated by these researchers by recording its CD spectrum, whereby a positive Cotton effect at 239.5 nm in the CD spectrum of **28** allowed the absolute configuration at C-1′ to be assigned.[26] As such, a positive Cotton effect at 230 nm in the CD spectrum of **26** suggests that the absolute configuration of C-1′ in **26** is the same as that reported in **28**. In defining the absolute configuration about C-1′ in **26**, it followed that the complete absolute configuration of this compound could be ascertained on the basis of the relative disposition of remaining centers to C-1′, as established previously by NOE experiments. Since **25** was confirmed to be the C-6′ epimer of **26**, the absolute configuration of **25** could also be tentatively assigned by inference to the absolute configuration established for **26**.

Compound **27** was also isolated as a colorless oil, and HRESIMS established the molecular formula as $C_{19}H_{34}O_3$ (333.2401 $[M + Na]^+$; calcd for $C_{19}H_{34}O_3Na$, 333.2406). The NMR data (Table 3) were very similar to the previously isolated compound **23**, indicating that **27** had the same linear terpene carbon skeleton.[15] Observation of a singlet at δ_H 3.22 correlating to a carbon at δ_C 49.7 in the HSQCAD and δ_C 77.6 in the HMBC experiments identified the presence of a methoxy moiety in **27** with HMBC correlations confirming its position in the structure of **27**. Compound **27** was confirmed to be the methylated analogue of compound **23**. The absolute configuration of the secondary alcohol moiety in **23** had been previously established by recording the CD of the hydrolysis product of **4** using the method of Nakanishi.[12,15] The coupling constant of the hydroxy methine in **23** (δ_H 3.36 dd J = 1.5, 10.5 Hz) was the same in **27** (δ_H 3.42 dd J = 1.5, 10.5 Hz), and as such the same configuration was assigned at C-13 in both **23** and **27**. It was thought that **27** could have been an artifact of the isolation procedure using methanol. In an effort to test this hypothesis a

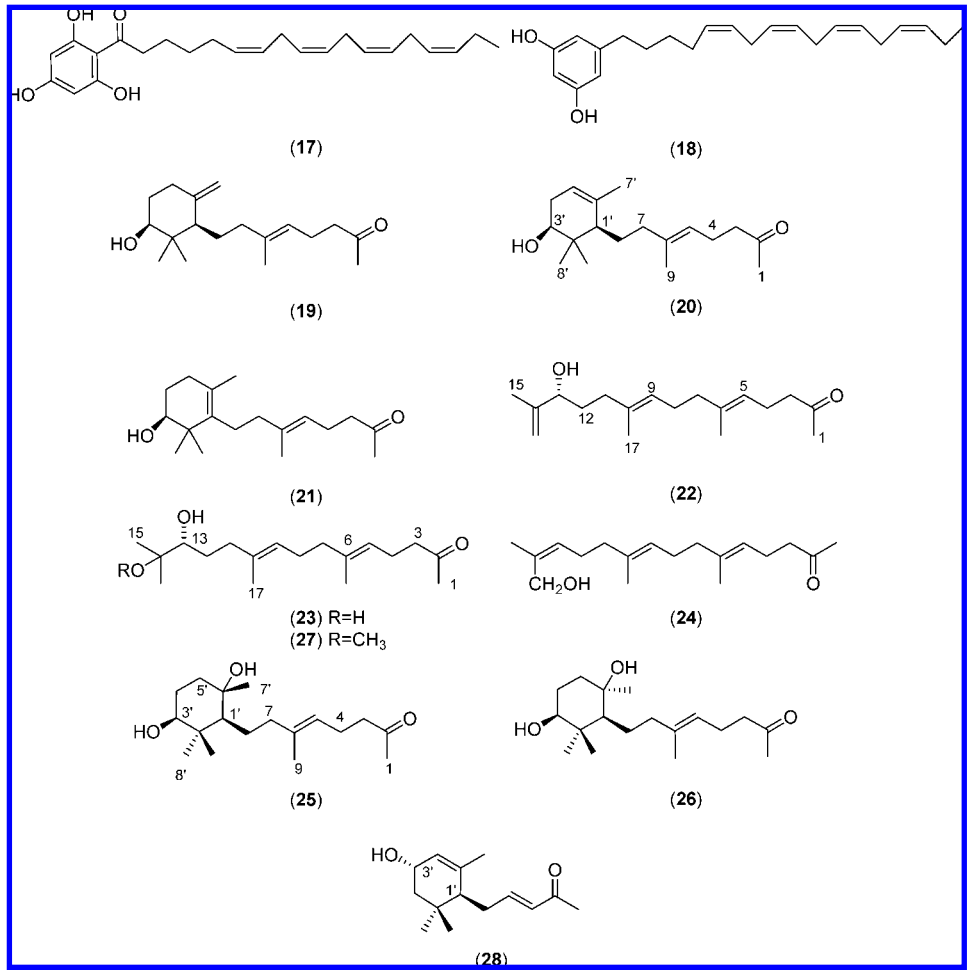


Table 1. NMR Data (500 MHz, $CDCl_3$) for Moniliforminol A (**25**)

position	δ_C[a], mult	δ_H (J in Hz)	gCOSY	gHMBC	selective 1D NOE
1	30.1, CH_3	2.13, s	3	2	
2	209.1, qC				
3	43.9, CH_2	2.48, t, (7.5)	1, 4	2, 4, 5	
4	22.6, CH_2	2.26, m	3, 5		
5	123.1, CH	5.08, dt, (1.0, 7.0)	4, 9	3, 4, 7, 9	
6	137.4, qC				
7	42.9, CH_2	2.08, s	8a, 8b	8	
8a	24.3, CH_2	1.55, m	7, 8b, 1′		
8b		1.42, m	7, 8a	1′	
9	16.3, CH_3	1.64, s	5	5, 6, 7	
1′	55.4, CH	1.08, t, (4.5)	8a, 8b	7, 8, 6′	7, 3′, 5′b, 9′
2′	41.1, qC[b]				
3′	78.4, CH	3.32, dd, (4.0, 11.0)	4′a		1′, 4′a, 5′b, 9′
4′a	29.3, CH_2	1.74, m	3	3′, 5′, 6′	
4′b		1.50, m	5′a		
5′a	41.1, CH_2[b]	1.77, m	4′b	3′, 6′	
5′b		1.48, m			
6′	73.5, qC				
7′	23.2, CH_3	1.15, s		1′, 5′, 6′	8′
8′	15.1, CH_3	0.79, s	9′	1′, 2′, 3′, 9′	7′, 9′
9′	28.3, CH_3	1.02, s	8′	1′, 2′, 3′, 8′	7, 8b, 3′, 8′
3′-OH	ND[c]				
6′-OH	ND				

[a] Carbon assignments based on HSQCAD and DEPT experiments. [b] Overlapped signals. [c] ND = not detected.

Table 2. NMR Data (500 MHz, $CDCl_3$) for Moniliforminol B (**26**)

position	δ_C[a], mult	δ_H (J in Hz)	gCOSY	gHMBC	selective 1D NOE
1	30.2, CH_3	2.14, s		2, 3	
2	209.0, qC				
3	43.9, CH_2	2.48, t, (7.0)	4, 5	2, 4, 5	
4	22.6, CH_2	2.26, q, (7.5)	3, 5	2, 3, 5, 6	
5	122.8, CH	5.11, dt, (1.5, 7.5)	3, 4, 9	4, 7, 9	
6	137.0, qC				
7	43.6, CH_2	2.03, m	8a, 8b, 9		
8a	24.8, CH_2	1.51, m	7	6′	
8b		1.44, m	7		
9	16.4, CH_3	1.66, s	5	5, 6, 7	
1′	53.2, CH	0.83, dd, (2.5, 4.5)			3′, 4′b, 7′, 9′
2′	40.5, qC				
3′	78.8, CH	3.25, dd, (4.5, 12.0)	4′a, 5′ b	8′, 9′	1′, 4′a, 9′
4′a	27.2, CH_2	1.80, m	3′, 4′b		3′, 4′b, 8′
4′b		1.62, m	4′a		
5′a	39.2, CH_2	1.68, m	5′b		5′b
5′b		1.52, m	3′, 5′a, 7′		
6′	72.7, qC				
7′	30.7, CH_3	1.17, s	5′b	1′, 5′, 6′	1′, 5′b, 9′
8′	14.9, CH_3	0.93, s	9′	1′, 2′, 3′, 9′	4′a
9′	27.1, CH_3	0.97, s	8′	1′, 2′, 3′, 8′	1′, 3′
3′-OH	ND[b]				
6′-OH	ND				

[a] Carbon assignments based on HSQCAD and DEPT experiments. [b] ND = not detected.

sample of **23** was left in methanol over a week, another was placed in methanol and heated, and the third was placed in methanol with three drops of formic acid and left for a week. Samples were then evaporated to dryness, resuspended in $CHCl_3$, and analyzed via GC/MS. Retention times of compounds **23** and **27** in $CHCl_3$ were used to monitor the possible formation of compound **27** from **23**. In the three varying experiments undertaken no evidence of **27** was apparent in any of the GC/MS analyses, and so it cannot be definitively concluded whether **27** is an artifact or an actual natural product. Due to the similarity of the coupling constant for the hydroxy methine in **27** and **23** as well as their co-occurrence, on

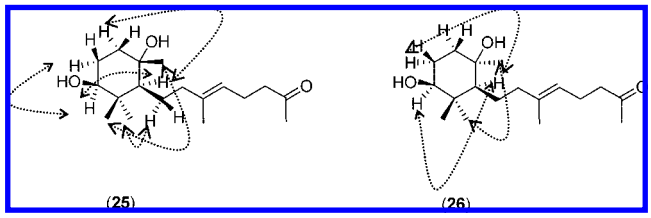


Figure 1. Key 1D NOE NMR enhancements for moniliforminols A (**25**) and B (**26**).

Table 3. NMR Data (500 MHz, $CDCl_3$) for Compound **27**

position	δc[a], mult	δ_H (J in Hz)	gCOSY	gHMBC
1	30.4, CH_3	2.13, s		2, 3
2	209.1, qC			
3	43.8, CH_2	2.46, t, (7.5)	4	1, 2, 4, 5
4	22.8, CH_2	2.26, m	3, 5	2, 3, 5, 6
5	122.7, CH	5.07, t, (6.5)	4	3, 4, 7, 18
6	136.6, qC			
7	39.8, CH_2	1.98, m	8	5, 6, 8, 18
8	26.8, CH_2	2.07, m	7, 9	7, 9, 10
9	124.7, CH	5.14, t, (6.5)	8	7, 8, 11, 17
10	135.4, qC			
11a	37.0, CH_2	2.27, m	11b, 12a	10 12, 13
11b		2.03, m	11a	12, 13, 17
12a	29.9, CH_2	1.50, m	11a, 12b	11
12b		1.39, m	12a, 13	11
13	76.6, CH	3.42, dd, (1.5, 10.5)	12b	11, 12, 14, 16
14	77.6, qC			
15	21.2, CH_3	1.12, s		13, 14, 16
16	19.0, CH_3	1.10, s		13, 14, 15
17	16.3, CH_3	1.61, s[b]		9, 10, 11a
18	16.3, CH_3	1.61, s[b]		5, 6, 7
19	49.3, CH_3	3.22, s		14
13-OH	ND[c]			

[a] Carbon assignments based on HSQCAD and DEPT experiments. [b] Overlapped signals. [c] ND = not detected.

biosynthetic grounds, the same C-13 absolute configuration has been assumed for compound **27**.

It is proposed that moniliforminols A (**25**) and B (**26**) could be biosynthetically produced as resultant acid-catalyzed cyclization products of compound **23**, which itself is derived from geranyl acetone. Compounds **19** to **21** are also cyclized terpenes, which are derivatives of moniliforminols A (**25**) and B (**26**) formed via dehydration reactions. The isolation of the more complex terpenoids **25** and **26** lends further support for *C. moniliformis* being one of the most developed species of the genera and are potential chemotaxonomic markers for this species.

Wells and co-workers have reported intriguing biological activity for the *Cystophora* spp.[15] The lipophilic extracts of the *Cystophora* spp. displayed *in vitro* antimicrobial activity against Gram-positive organisms, and the compounds responsible for the activity were established to be phloroglucinol (**17**), resorcinol (**18**), and δ-tocotrienol (**15**).[15] On the contrary *C. moniliformis* had been reported to produce metabolites such as **1** to **14**, for which the lipophilic extract showed no *in vitro* antimicrobial activity, but it did display weak anticonvulsant activity, for which the major terpenoids, including the farnesylacetone derivatives **3** and **4**, were found to be responsible.[15,32] According to Wells et al. these terpenoid ketones have also been suggested to have roles as feeding deterrents, where the crude extracts of *C. moniliformis* were reported to display juvenile hormone activity.[12,19] The farnesylacetone terpenoid **1** and its hexahydro derivative **2** were analyzed in the *Galleria* wax test and were found to be not as active as the crude extract.[12,19]

Compounds **21** to **27** displayed no appreciable antitumor activity (IC_{50} of >40 μM when tested at 1 mg/mL) or antifungal activity (1 mm zone of inhibition detected against *Trichophyton mentagrophytes*). The mixture of compounds **19** and **20**, obtained in a 3:1 ratio, displayed moderate antitumor activity (IC_{50} of 45 μM when tested at 1 mg/mL) and moderate antifungal activity (4 mm zone of inhibition detected against *Trichophyton mentagrophytes*). All other isolated compounds showed no inhibition of *T. mentagrophytes* when tested at 1 mg/mL. Recently the antiviral assays conducted at the University of Canterbury were phased out, which meant that no antiviral assessment of the isolated compounds could be carried out.

The linear terpene **24** and related polyprenyl ketones have been previously described as synthetically prepared derivatives that have been patented for their antiulcer activity and hypotensive activity.[22] This represents the first report of compound **24** occurring as a natural product.

Experimental Section

General Experimental Procedures. All organic solvents used were analytical reagent (AR or GR), UV spectroscopic, or HPLC grades with milli-Q water also being used. Optical rotations were carried out using a 1.2 mL cell on a Jasco DIP-1000 digital polarimeter, set to the Na 589 nm wavelength. UV/vis spectra were recorded on a Varian CARY 50 Bio spectrophotometer, using EtOH. In addition a UV profile was obtained from the HPLC (PDA detection) by extraction of the 2D contour plot. CD spectra were obtained on a Jasco 815 spectrometer in EtOH. IR spectra were recorded as a film using a NaCl disk on a Perkin-Elmer Spectrum One FTIR spectrometer. ^1H (500 MHz), ^{13}C (125 MHz), and single irradiation NOE spectra were acquired in $CDCl_3$ on a 500 MHz Varian INOVA spectrometer with referencing to residual solvent signals (δ 7.26 and 77.0 ppm). Two-dimensional NMR experiments recorded included gCOSY, gHSQCAD, and gHMBC experiments. ESI mass spectra were obtained on a Micromass Platform II mass spectrometer equipped with a LC-10AD Shimadzu solvent delivery module (50% CH_3CN/H_2O at a flow rate of 0.1 mL/min) in both the positive and negative ionization modes using cone voltages between 20 and 30 V. HRESIMS was carried out on either an Agilent G1969A LC time-of-flight (TOF) system (ESI operation conditions of 8 L/min N_2, 350 °C drying gas temperature, and 4000 V capillary voltage) equipped with an Agilent 1100 Series LC solvent delivery module (50% CH_3OH/H_2O with 0.1% acetic acid at a flow rate of 0.3 mL/min) or an Agilent 6200 Series TOF system (ESI operation conditions of 8 L/min N_2, 350 °C drying gas temperature, and 4000 V capillary voltage) equipped with an Agilent 1200 Series LC solvent delivery module (100% CH_3OH at a flow rate of 0.3 mL/min) in either the negative and positive ionization modes (in all cases the instruments were calibrated using the Agilent Tuning Mix using purine as the reference compound). GC-MS analysis was carried out on a Varian Saturn 2200 GC-MS/MS and Varian CP-3800 gas chromatograph using a Varian VF-5 ms (30 m × 0.25 mm i.d.) GC column with a constant column flow of 1.0 mL/min. A temperature program starting at 60 °C (held for 1 min), which is then ramped at 10 °C/min to 260 °C and held at this temperature for 5 min, was utlized. GC parameters included an injector temperature of 250 °C and detector temperature of 260 °C, with injector port set to standard split/splitless mode and a total run time of approximately 25 min. The mass spectrometer was set to the EI mode with a trap temperature of 200 °C and a mass scan range between m/z 40 and 450. TLC was performed on precoated aluminum-backed silica gel TLC plates (Merck silica gel 60 F_{254}) using the solvent system 65:25:4 $CHCl_3/CH_3OH/H_2O$, visualized at 254 and 365 nm and further developed using (A) iodine vapor and (B) a ninhydrin dip consisting of 0.3 g of ninhydrin in 100 mL of *n*-butanol and 3 mL of HOAc. Silica gel flash chromatography was carried out with Merck silica gel (60 mesh) using N_2 and a 20% stepwise solvent elution from 100% hexanes to 100% CH_2Cl_2 to 100% EtOAc and finally to 100% CH_3OH. Gel permeation chromatography was performed using Sephadex LH-20 (Sigma) with 100% CH_3OH as the eluant. All analytical HPLC analyses were performed on a Dionex P680 solvent delivery system equipped with a PDA100 UV detector (operated using "Chromeleon" software). Analytical HPLC analyses were run using either a gradient method (0 min 10% CH_3CN/H_2O; 2 min 10% CH_3CN/H_2O; 14 min 75% CH_3CN/H_2O; 24 min 75% CH_3CN/H_2O; 26 min 100% CH_3CN; 30 min 100% CH_3CN; 32 min 10% CH_3CN/H_2O; and 40 min 10% CH_3CN/H_2O) or an isocratic method (either 60% CH_3CN/H_2O or 65% CH_3CN/H_2O) on a Phenomenex Prodigy ODS (3) C_{18} 100 Å 250 × 4.6 (5 μm) and on a Phenomenex Luna ODS (3) C_{18} 100 Å 250 × 4.6 (5 μm) column at a flow rate of 1.0 mL/min. All

semipreparative HPLC was carried out on a Varian Prostar 210 (solvent delivery module) equipped with a Varian Prostar 335 PDA detector using STAR LC WS version 6.0 software using an isocratic method (65% CH_3CN/H_2O) and a Phenomenex Prodigy ODS (3) 100 Å C_{18} 250 × 10 (5 μm) column at a flow rate of 3.5 mL/min.

Biological Evaluation and Details of Assays. Extracts of the alga were evaluated (tested at 50 mg/mL) in a number of biological assays including against a P388 murine leukemia cell line (antitumor assay), against *Herpes simplex* and *Polio* viruses (antiviral assays), and against a number of bacteria and fungi (antimicrobial assays) at the University of Canterbury, Christchurch, New Zealand. Moderate antitumor activity was observed for the alga extract (38532 ng/mL at 50 mg/mL). In addition the extract displayed cytotoxic activity against the *H. simplex* and the *Polio* virus as well as moderate antimicrobial activity (a 4 mm zone of inhibition was detected against *Trichophyton mentagrophytes*). No activity was observed for the extract against *Eschericha coli*, *Pseudomonas aeruginosa*, *Candida albicans, Bacillus subtilis*, or *Cladosporium resinae*.

Antitumor Assay (P388 murine leukemia cell line). For the antitumor assay a 2-fold dilution series of the crude extract as well as compounds **19** to **27** were incubated for 72 h with P388 (murine leukemia) cells. The concentration of sample required to reduce the P388 cell growth by 50% (comparative to control cells) was determined using the absorbance values obtained when the yellow dye MTT tetrazolium is reduced by healthy cells to the purple color MTT formazan and is expressed as an IC_{50}, in ng/mL.

Antiviral Assays (herpes simplex virus and polio virus). The crude extract was pipetted onto 6 mm diameter filter paper disks and the solvent evaporated. The disk was then placed directly onto BSC-1 cells (African green monkey kidney), infected with either the DNA *Herpes simplex* virus type 1 (ATCC VR-733) or the RNA *Polio* virus type 1 (ATCC VR-192) and then incubated. The assays were examined after 24 h using an inverted microscope for the size of antiviral or viral inhibition and/or cytotoxic zones and the type of cytotoxicity. Recently, the University of Canterbury has phased out these antiviral assays.

Antimicrobial Assays. Bacteria or fungi at a known concentration were mixed with Mueller Hinton or potato dextrose agar and poured into Petri dishes so that after incubation a "lawn" of bacteria/fungi grew over the dish. The crude extract as well as compounds **19** to **27** were pipetted onto 6 mm diameter filter paper disks and their solvents evaporated. These disks were then placed onto the prepared seeded agar dishes (with appropriate solvent and positive controls) and incubated. Active antimicrobial samples displayed a zone of inhibition outside the disk, which was measured in millimeters as the radius of inhibition for each bacteria/fungi. The six organisms were *Eschericha coli* (G−ve ATCC 25922), *Bacillus subtilis* (G+ve ATCC 19659), and *Pseudomonas aeruginosa* (G−ve ATCC27853) for the bacteria and *Candida albicans* (ATCC 14053), *Trichophyton mentagrophytes* (ATCC 28185), and *Cladosporium resinae* for the fungi. Since the completion of these studies the University of Canterbury has phased out these antimicrobial assays.

Marine Alga Material. The marine brown alga (*Cystophora moniliformis*) was collected by scuba on April 30, 2004, from Port Phillip Bay, Victoria, Australia. The alga was identified by Dr. Gerald Kraft (Honorary Principal Fellow), Faculty of Science, School of Botany, University of Melbourne, Australia. A voucher specimen designated the code 2004-09 is deposited at the School of Applied Sciences (Discipline of Applied Chemistry), RMIT University.

Extraction and Isolation. The alga (20 g) was extracted with 3:1 CH_3OH/CH_2Cl_2 (700 mL), and the crude extract was decanted and concentrated under reduced pressure and subsequently sequentially solvent partitioned into CH_2Cl_2-, CH_3OH-, and water-soluble extracts. The CH_2Cl_2 extract was fractionated using flash silica gel column chromatography (20% stepwise elution from hexanes to CH_2Cl_2 to EtOAc and finally to CH_3OH). The 40:60 CH_2Cl_2/EtOAc silica gel column fraction was subjected to repeated gel permeation chromatography (Sephadex LH-20 using 100% CH_3OH) followed by reversed-phase HPLC (65% CH_3CN/H_2O) to yield compound **23** (12 mg, 0.14%), moniliforminol A (**25**) (6 mg, 0.07%), and moniliforminol B (**26**) (6.5 mg, 0.08%). The 80:20 CH_2Cl_2/EtOAc silica gel column fraction was also subjected to gel permeation chromatography (Sephadex LH-20 using 100% CH_3OH) and then reversed-phase HPLC (65% CH_3CN/H_2O) to yield a 3:1 mixture of compounds **19** and **20** (8 mg, 0.09%), compound **21** (5.0 mg, 0.06%), compound **22** (8 mg, 0.09%), compound **24** (7 mg, 0.08%), and compound **27** (7 mg, 0.08%).

Compound 19 [(*E*)-8-(1*R*,5*S*)-5-hydroxy-2,6,6-trimethylcyclohex-2-enyl)-6-methyloct-5-en-2-one]: (major compound) colorless, viscous oil in a mixture with compound **20**; UV profile from HPLC (CH_3CN/H_2O) 210 nm; IR has been previously reported;[15] partial ^{1}H NMR (500 MHz, $CDCl_3$) extrapolated from a mixture of **19** and **20** δ 5.04 (1H, t, J = 7.0 Hz, H-5), 4.86 (1H, s, H-7′a), 4.58 (1H, s, H-7′b), 3.41, (1H, dd, J = 4.5, 10.0 Hz, H-3′), 2.46 (2H, m, H-3), 2.26 (4H, q, J = 7.5 Hz, H-4), 2.14 (6H*, s, H-1), 1.61 (3H, s, H-9), 1.02 (3H, s, H-8′), 0.71 (3H, s, H-9′); ^{13}C NMR has been previously reported;[15] ESIMS (positive mode) m/z 279 $[M + H]^+$. (*Signal overlapped with methyl of compound **20** and 3′-OH not detected.)

Compound 20 [(*E*)-8-(1*R*,3*S*)-3-hydroxy-2,2-dimethyl-6-methyl-enecyclohexyl)-6-methyloct-5-en-2-one]: (minor compound) colorless, viscous oil in a mixture with compound **19**; IR has been previously reported;[15] UV profile from HPLC (CH_3CN/H_2O) 210 nm; partial ^{1}H NMR (500 MHz, $CDCl_3$) extrapolated from a mixture of **20** and **19** δ 5.23 (1H, m, H-5′), 5.09 (1H, t, J = 6.8 Hz, H-5), 3.46, (1H, dd, J = 5.5, 8.0 Hz, H-3′), 2.47 (2H, m, H-3), 2.32 (4H, dt, J = 5.0, 13.0 Hz, H-8), 2.14 (6H*, s, H-1), 1.70 (3H, bs, H-9), 1.63 (3H, s, H-7′), 0.96 (3H, s, H-8′), 0.82 (3H, s, H-9′); ^{13}C NMR has been previously reported;[15] ESIMS (positive mode) m/z 279 $[M + H]^+$. (*Signal overlapped with methyl of compound **19** and 3′-OH not detected.)

Compound 21, (*S*,*E*)-8-(5-hydroxy-2,6,6-trimethylcyclohex-1-enyl)-6-methyloct-5-en-2-one: colorless, volatile oil; due to its volatility, an optical rotation of this compound could not be carried out, as most of the mass was lost upon drying; UV (EtOH) λ_{max} (log ε) 205 nm (4.06); IR has been previously reported;[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (1H, t, J = 7.2 Hz, H-5), 3.50 (1H, m, H-3′), 2.47 (2H, t, J = 7.5 Hz, H-3), 2.27 (2H, q, J = 7.0 Hz, H-4), 2.15 (3H, s, H-1), 2.04 (4H, m, H-8 and H-5′)*, 2.01 (2H, m, H-7), 1.80 (1H, m, H-4′a), 1.68 (1H, m, H-4′b), 1.66 (3H, s, H-9), 1.61 (3H, s, H-7′), 1.07 (3H, s, H-8′), 1.01 (3H, s, H-9′); ^{13}C (125 MHz, $CDCl_3$) 209.0 (C, C-2), 137.4 (C, C-6), 135.6 (C, C-1′), 126.6 (C, C-6′), 122.3 (CH, C-5), 76.3 (CH, C-3′), 43.9 (CH_2, C-3), 40.2 (C, C-7)*, 40.2 (CH_2, C-2′)*, 30.1 (CH_3, C-1), 29.9 (CH_2, C-5′), 28.1 (CH_2, C-8), 26.6 (CH_2, C-4′), 26.5 (CH_3, C-8′), 22.6 (CH_2, C-4), 21.9 (CH_3, C-9′), 19.7 (CH_3, q, C-7′) and 16.4 (CH_3, C-9); due to its volatility, a mass spectrum of this compound could not be carried out, as most of the mass was lost upon drying. (*Overlapped signals and 3′-OH not detected.)

Compound 22, (5*E*,9*E*)-13-hydroxy-6,10,14-trimethylpentadeca-5,9,14-trien-2-one: colorless, viscous oil; $[\alpha]^{21}_D$ −36 (*c* 0.02, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 205 nm (3.72); IR has been previously reported;[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (1H, t, J = 7.0 Hz, H-9), 5.07 (2H, t, J = 7.0 Hz, H-5), 4.94 (1H, bs, H-16a), 4.84 (1H, d, J = 1.24 Hz, H-16b), 4.04 (2H, t, J = 6.3 Hz, H-13), 2.60 (1H, bs, 13-OH), 2.46 (2H, t, J = 7.5 Hz, H-3), 2.26 (2H, q, J = 7.0 Hz, H-4), 2.14 (3H, s, H-1), 2.08 (2H, m, H-8), 2.02 (2H, m, H-11), 1.99 (2H, m, H-7), 1.73 (3H, s, H-15), 1.63 (2H, m, H-12), 1.61 (6H, s, H-17 and H-18)*; ^{13}C (125 MHz, $CDCl_3$) 208.3 (C, C-2), 147.8 (C, C-14), 136.6 (C, C-10), 135.8 (C, C-6), 124.8 (CH, C-9), 123.0 (CH, C-5), 111.3 (CH_2, C-16), 75.8 (CH, C-13), 43.9 (CH_2, C-3), 39.8 (CH_2, C-7), 35.9 (CH_2, C-11), 33.2 (CH_2, C-12), 29.8 (CH_3, C-1), 26.5 (CH_2, C-8), 22.6 (CH_2, C-4), 18.0 (CH_3, C-15), 16.1 (CH_3, C-17)*, 16.1 (CH_3, C-18)*; ESIMS (positive mode) m/z 279 $[M + H]^+$, 261 $[(M + H) - H_2O]^+$. (*Overlapped signals)

Compound 23, (*R*,5*E*,9*E*)-13,14-dihydroxy-6,10,14-trimethylpentadeca-5,9-dien-2-one: colorless, viscous oil; $[\alpha]^{25}_D$ −13.9 (*c* 0.094, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 207 nm (3.82); IR has been previously reported;[15] ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (1H, t, J = 7 Hz, H-9), 5.07 (2H, dt, J = 7 Hz, H-5), 3.36 (1H, dd, J = 1.5, 10.5 Hz, H-13), 2.47 (2H, t, J = 7.5 Hz, H-3), 2.27 (2H, m, H-4), 2.23 (1H, m, H-11a), 2.13 (3H, s, H1), 2.09 (3H, m, H-8, H-11b)*, 2.00 (2H, t, J = 7.5 Hz, H-7), 1.60 (7H, m, H-12a, H-17, H-18)*, 1.41 (1H, m, H-12b), 1.20 (3H, s, H-15), 1.16 (3H, s, H-16); ^{13}C (125 MHz, $CDCl_3$) δ 209.2 (C, C-2), 136.1 (C, C-6), 134.9 (C, C-10), 124.8 (CH, C-9), 122.8 (CH, C-5), 78.1 (CH, C-13), 72.9 (C, C-14), 43.7 (CH_2, C-3), 39.5 (CH_2, C-7), 36.7 (CH_2, C-11), 29.9 (CH_3, C-1), 29.6 (CH_2, C-12), 26.4 (CH_2, C-8), 26.3 (CH_3, C-15), 23.3 (CH_3, C-16), 22.4 (CH_2, C-4), 15.9 (CH_3, C-17)*, 15.9 (CH_3, C-18)*; GC-EI/MS m/z (relative intensity) 279 (12) $[M\text{-}OH, C_{18}H_{31}O_2]^+$, 261 (6), 243 (35), 237 (11) $[C_{15}H_{25}O_2]^+$, 219 (6), 201 (43), 175 (28), 161 (46), 134 (21), 123 (22), 109 (30), 95 (50), 81 (20), 67 (19), 59 (21), 43 (100); ESIMS (negative mode) m/z 295 $[M - H]^-$. (*Overlapped signals and 13-OH not detected.)

Compound 24, (5*E*,9*E*,13*Z*)-15-hydroxy-6,10,14-trimethylpentadeca-5,9,13-trien-2-one: colorless, viscous oil; UV (EtOH) λ_{max} (log ε) 205 nm (3.64); IR (film) ν_{max} 3402, 2918, 1713, 1463, 1381, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.27 (1H, t, $J = 7.5$ Hz, H-13), 5.08 (1H, m, H-9), 5.07 (1H, m, H-5), 4.11 (2H, s, H-16), 3.15 (1H, bs, 16-OH), 2.46 (2H, t, $J = 7.0$ Hz, H-3), 2.26 (2H, q, $J = 7.0$ Hz, H-4), 2.14 (3H, s, H-1), 2.13 (2H, m, H-12), 2.07 (2H, q, $J = 7.0$ Hz, H-8), 1.98 (2H, m, H-11), 1.97 (2H, m, H-7), 1.79 (3H, s, H-15), 1.61 (3H, s, H-18), 1.59 (3H, s H-17); ^{13}C (125 MHz, $CDCl_3$) 209.3 (C, C-2), 136.7 (C, C-6), 135.1 (C, C-10), 134.6 (C, C-14), 128.6 (CH, C-13), 124.9 (CH, C-9), 122.9 (CH, C-5), 61.8 (CH_2, C-16), 44.1 (CH_2, C-3), 39.9 (CH_2, C-7)*, 39.9 (CH_2, C-11)*, 30.2 (CH_3, C-1), 26.7 (CH_2, C-8), 26.5 (CH_2, C-12), 22.6 (CH_2, C-4), 21.6 (CH_3, C-15), 16.3 (CH_3, C-17), 16.2 (CH_3, C-18); ESIMS (positive mode) *m/z* 279 $[M + H]^+$; HRESIMS *m/z* 279.2311 $[M + H]^+$ (calcd for $C_{18}H_{33}O_3$, 279.2324) and *m/z* 301.2141 $[M + Na]^+$ (calcd for $C_{18}H_{32}O_3Na$, 301.2143). (*Overlapped signals)

Moniliforminol A (25), (*E*)-8-((1S,3S,6S)-3,6-dihydroxy-2,2,6-trimethylcyclohexyl)-6-methyloct-5-en-2-one: colorless, viscous oil; $[\alpha]^{25}_D$ +12.75 (*c* 0.032, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 200 nm (3.96); IR (film) ν_{max} 3401, 2925, 1709, 1589, 1458, 1347, 1309, 1162 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) see Table 1; GC-EI/MS *m/z* (relative intensity) 281 (1) $[M - CH_3, C_{17}H_{29}O_3]^+$, 211 (3), 182 (7), 169 (9), 157 (13) $[C_9H_{17}O_2]^+$, 140 (8), 127 (6), 115 (23), 98 (18), 83 (7), 73 (13), 53 (2), 43 (100); ESIMS (positive mode) *m/z* 318.9 $[M + Na]^+$; HRESIMS *m/z* 319.2252 $[M + Na]^+$ (calcd for $C_{18}H_{32}O_3Na$, 319.2249).

Moniliforminol B (26) (*E*)-8-(1S,3S,6R)-3,6-dihydroxy-2,2,6-trimethylcyclohexyl)-6-methyloct-5-en-2-one: colorless, viscous oil; $[\alpha]^{25}_D$ −8.0 (*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 200 nm (4.00); CD (EtOH) 230 nm [$\theta = +22°$ and $\Delta\varepsilon$ +6.67]; IR (film) ν_{max} 3369, 2927, 1714, 1589, 1456, 1371, 1347, 1307, 1163 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) see Table 2; GC-EI/MS *m/z* (relative intensity) 296 (5) $[M]^+$, 264 (33) $[C_{17}H_{28}O_2]^+$, 249 (1), 235 (8), 222 (14), 180 (12), 166 (10), 157 (1) $[C_9H_{17}O_2]^+$, 137 (13), 123 (19), 111 (26) $[C_7H_{11}O]^+$, 96 (40), 83 (63), 67 (57), 55 (100); ESIMS (positive mode) *m/z* 318.9 $[M + Na]^+$; HRESIMS *m/z* 319.2251 $[M + Na]^+$ (calcd for $C_{18}H_{32}O_3Na$, 319.2249).

Compound 27, (*R*,5*E*,9*E*)-13-hydroxy-14-methoxy-6,10,14-trimethylpentadeca-5,9-dien-2-one: colorless, viscous oil; $[\alpha]^{25}_D$ −46.8 (*c* 0.016, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 201 nm (3.52); UV profile from HPLC (CH_3CN/H_2O) 210 nm; IR (film) ν_{max} 3468, 2928, 1716, 1445, 1362, 1151, 1078 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) see Table 3; GC-EI/MS *m/z* (relative intensity) 278 (1) $[C_{18}H_{30}O_2]^+$, 243 (5), 237 (1), 217 (3), 201 (16), 189 (3), 175 (4), 161 (9), 135 (10), 121 (6), 107 (12), 93 (8), 81 (14), 73 (100), 55 (6), 43 (55); ESIMS (positive mode) *m/z* 333.3 $[M + Na]^+$; HRESIMS *m/z* 333.2401 $[M + Na]^+$ (calcd for $C_{19}H_{34}O_3Na$, 333.2406).

Acknowledgment. The Marine and Terrestrial Natural Product (MATNAP) research group thanks Dr. G. Kraft (University of Melbourne, Victoria, Australia) for the alga identification; Mr. R. Watson from the Victorian Marine Science Consortium (VMSC) for the collection of the alga; Ms. G. Ellis (University of Canterbury, Christchurch, New Zealand) for the biological testing; Ms. S. Duck (School of Chemistry, Faculty of Science, Monash University) for HRMS analyses; Dr. D. Tew (Department of Pathology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne) for the CD analysis; Ms. F. Charalambous and Mr. N. C. Wee for assistance with some of the fractionation; Dr. I. Van Altena for informative discussions on *C. moniliformis*; and the Victorian Institute for Chemical Sciences (VICS) for financial support.

References and Notes

(1) Womersley, H. B. W. *Aust. J. Bot.* **1964**, *12*, 53–110.
(2) Amico, V. *Phytochemistry* **1995**, *39*, 1257–1279.
(3) Blunt, J. W.; Munro, M. H. *MarinLit Database* 2007, Department of Chemistry, University of Canterbury, Christchurch, New Zealand.
(4) Laird, D. W.; Poole, R.; Wikstrom, M.; Van Altena, I. A. *J. Nat Prod.* **2007**, *70*, 671–674.
(5) Valls, R.; Piovetti, L. *Biochem. Syst. Ecol.* **1995**, *23*, 723–745.
(6) Bian, B.; Van Altena, I. A. *Aust. J. Chem.* **1998**, *51*, 1157–1165.
(7) Capon, R. J.; Ghisalberti, E. L.; Jefferies, P. R. *Phytochemistry* **1981**, *20*, 2598–2600.
(8) Glombitza, K.-W.; Hauperich, S. *Phytochemistry* **1997**, *46*, 735–740.
(9) Gregson, R. P.; Daly, J. J. *Aust. J. Chem.* **1982**, *35*, 649–657.
(10) Gregson, R. P.; Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Aust. J. Chem.* **1977**, *30*, 2527–2532.
(11) Kazlauskas, R.; King, L.; Murphy, P. T.; Warren, R. G.; Wells, R. J. *Aust. J. Chem.* **1981**, *34*, 439–447.
(12) Kazlauskas, R.; Murphy, P. T.; Wells, R. J. *Experientia* **1978**, *34*, 156–157.
(13) Koch, M.; Gregson, R. P. *Phytochemistry* **1984**, *23*, 2633–2637.
(14) Laird, D. W.; Van Altena, I. A. *Phytochemistry* **2006**, *67*, 944–955.
(15) Ravi, B. N.; Murphy, P. T.; Lidgard, R. O.; Warren, R. G.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 171–182.
(16) Sailler, B.; Glombitza, K.-W. *Nat. Toxins* **1999**, *7*, 57–62.
(17) Sailler, B.; Glombitza, K.-W. *Phytochemistry* **1999**, *50*, 869–881.
(18) Sailler, B.; Glombitza, K. W. *Nat. Toxins* **1999**, *7*, 57–62.
(19) Van Altena, I. A. *Aust. J. Chem.* **1988**, *41*, 49–56.
(20) Amico, V.; Oriente, G.; Piattelli, M.; Ruberto, G.; Tringali, C. *Phytochemistry* **1980**, *19*, 2759–2760.
(21) Teixeira, V. L.; Kelecom, A. *Sci. Total Environ.* **1986**, *58*, 109–115.
(22) Sato, A.; Nakamjima, K.; Takahara, Y.; Kijima, S.; Yamatsu, I.; Suzuki, K.; Suzuki, T.; Nakamura, T. Eur. Pat. Appl. 50853, 1982.
(23) Kasparek, S. *Vitamin E: A Comprehensive Treatise*; Machlin, L. J., Ed.; Marcel Dekker: New York, 1980; Chapter 2: Chemistry of Tocopherols and Tocotrienols, pp 7−65.
(24) Wehrli, F. W.; Nishida, T. *Fortschr. Chem. Org. Naturst.* **1979**, *36*, 1–229.
(25) Wu, H.; Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2495–2504.
(26) Machida, K.; Kikuchi, M. *Phytochemistry* **1996**, *41*, 1333–1336.
(27) Sun, Y.; Zhan, Y.-C.; Sha, Y.; Pei, Y.-H. *J. Asian Nat. Prods. Res.* **2007**, *9*, 321–325.
(28) Mori, K. *Tetrahedron* **1974**, *30*, 1065–1072.
(29) Miyake, Y.; Hideyuki, I.; Yoshida, T. *Can. J. Chem.* **1997**, *75*, 734–741.
(30) Eschenmoser, W.; Uebelhart, P.; Eugster, C. H. *Helv. Chim. Acta* **1979**, *62*, 2534–2538.
(31) Baumeler, A.; Eugster, C. H. *Helv. Chim. Acta* **1991**, *74*, 469–486.
(32) Spence, I.; Jamieson, D. D.; Taylor, K. M. *Experientia* **1979**, *35*, 238–239.

NP800201C