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Meroditerpenoids from the southern Australian marine brown alga *Sargassum fallax*

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ABSTRACT

Chemical investigation of the southern Australian marine brown alga *Sargassum fallax* resulted in the isolation of three meroditerpenoids fallahydroquinone, fallaquinone and fallachromenoic acid together with the previously reported compounds sargaquinone [isolated and identified in a mixture with sargaquinoic acid], sargahydroquinoic acid, sargaquinoic acid and sargachromenol. As a result of this study the complete 2D NMR characterisation for sargaquinoic acid and sargahydroquinoic acid can now be reported for the first time. All structures were elucidated by detailed spectrometric analysis. Sargaquinoic acid and sargahydroquinoic acid displayed moderate antitumour activity.

1. Introduction

The phytochemistry of an estimated 62 species of the *Sargassum* genus (Sargassaceae, Fucales) has been reported to date (Blunt and Munro, 2007; Blunt et al., 2008). *Sargassum* sp. are found throughout tropical and subtropical areas of the world and are reported to produce metabolites of structural classes such as plastoquinones (Segawa and Shirahama, 1987; Mori et al., 2005; Ishitsuka et al., 1979), chromanols (Kato et al., 1975), chromenes (Jang et al., 2005; Kikuchi et al., 1975), steroids (Tang et al., 2002a) and glycerides (Tang et al., 2002b). The meroditerpenoids (plastoquinones, chromanols and chromenes), consisting of a polyprenyl chain attached to a hydroquinone ring moiety, are present in many marine organisms such as coelenterates, fish, macroalgae, sponges and tunicates (Jang et al., 2005). Brown algae (division Phaeophyta) produce myriad secondary metabolites of this class, making meroditerpenoids such as sargaquinone (**1**), sargaquinoic acid (**2**) and sargahydroquinoic acid (**3**) just one of the representative groups of secondary metabolites produced by these organisms (Jang et al., 2005). Plastoquinones from the *Sargassum* genus generally adopt the same structural skeleton and differs primarily in the linear terpene chain moiety (Blunt and Munro, 2007). The linear chain component of plastoquinones differ mostly in the positions of the hydroxy and carbonyl groups, such as in compounds (**4**)–(**7**) (Blunt and Munro, 2007; Mori et al., 2005; Komai et al., 2006).

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, *Sargassum fallax*, collected from Port Phillip Bay, Victoria, Australia. We describe here the isolation and structure determination of three new meroditerpenoids fallahydroquinone (**8**), fallaquinone (**9**) and fallachromenoic acid (**10**), together with the known meroterpenoids sargaquinone (**1**) [identified in a mixture with sargaquinoic acid (**2**)], sargaquinoic acid (**2**), sargahydroquinoic acid (**3**) and sargachromenol (**11**). The complete 2D NMR characterisation for **2** and **3** are reported for the first time and additional spectroscopic characterisation data is reported for **11**.

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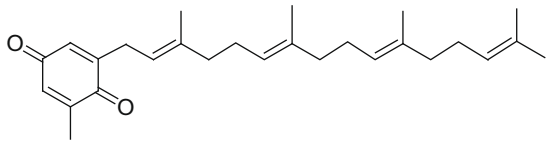

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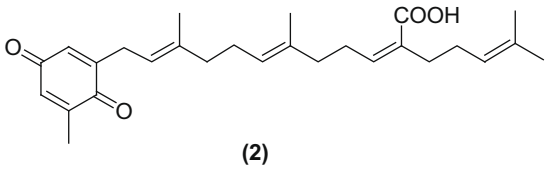

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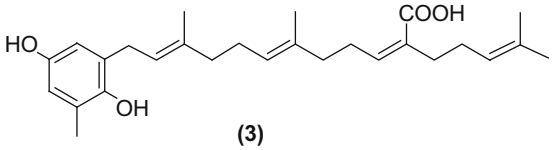

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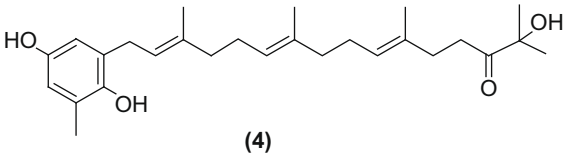

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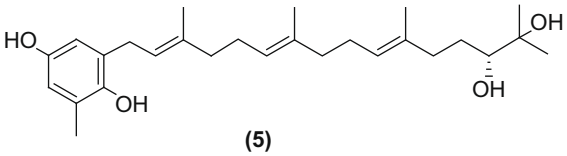

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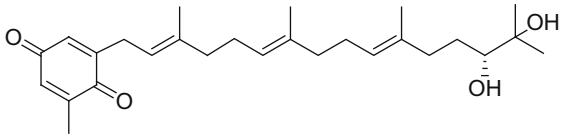

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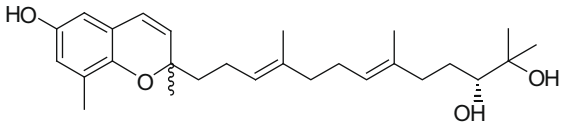

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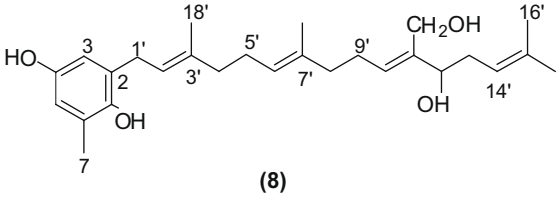

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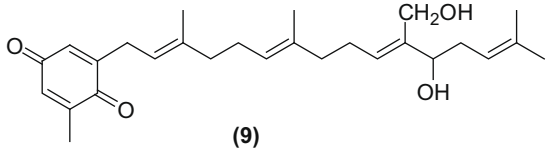

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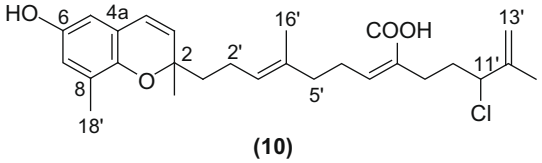

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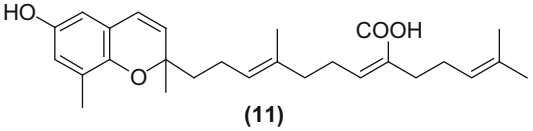

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2. Results and discussion

The alga was extracted with 3:1 MeOH/DCM, evaporated under vacuum, and then subsequently portioned into DCM, MeOH and water fractions, respectively. The DCM partition was fractionated by silica gel flash chromatography and the 100% EtOAc and 40:60 DCM/EtOAc column fractions were each subjected to repeated gel permeation chromatography (Sephadex LH-20, using 100% MeOH). Final purification of each of these fractions was achieved by reversed phase HPLC to yield fallahydroquinone (**8**) from the 100% EtOAc silica gel column fraction and sargaquinoic acid (**2**), sargahydroquinoic acid (**3**), fallaquinone (**9**), sargachromenol (**11**) from the 40:60 DCM/EtOAc fraction. Sargaquinone (**1**) and fallachromenoic acid (**10**) were subsequently isolated directly from the DCM extract by reversed phased HPLC.

Sargaquinone (**1**) was isolated as a mixture with sargaquinoic acid (**2**) in a ratio of 1:20 and was identified on the basis of a direct comparison of the ^{1}H and ^{13}C NMR data to the literature (Amico et al., 1985). Sargahydroquinoic acid (**3**) had been previously reported but identified solely on the basis of ^{1}H NMR assignments and mass spectrometry while sargaquinoic acid (**2**) had been previously identified based on a combination of ^{1}H and ^{13}C NMR assignments and mass spectrometry (Segawa and Shirahama, 1987; Kusumi et al., 1979; Seo et al., 2006, 2007). The re-isolation of **2** and **3** in this study provided the opportunity to report the first ^{13}C NMR data for sargahydroquinoic acid (**3**) as well as complete unequivocal structural assignment of both compounds using 2D NMR spectroscopy.

Fallahydroquinone (**8**) was isolated as a pale yellow oil, for which high resolution ESIMS established the molecular formula as $C_{27}H_{40}O_4$ (451.2812 $[M+Na]^+$, calcd for $C_{27}H_{40}O_4Na$, 451.2824), possessing nine degrees of unsaturation. The IR spectrum supported the presence of hydroxy (3369 cm^{-1}) and, olefinic moieties (1441 cm^{-1}) together with a ketone stretch (w 1653 cm^{-1}). The presence of the weak carbonyl stretch was indicative of a conver-

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignment of fallahydroquinone (**8**) in $CDCl_3$.

Position	δ_H (*J* in Hz)	δc[a], mult	gCOSY	gHMBC
1		146.0 *s*		
2		125.4 *s*		
3	6.48 *d* (3.0)	114.1 *d*	5	1, 4, 5, 1′
4		149.3 *s*		
5	6.51 *d* (3.0)	115.6 *d*	3, 7	1, 3, 4, 7
6		127.8 *s*		
7	2.18 *s*	16.1[b] *q*	5	1, 5, 6
1′	3.28 *d* (7.0)	29.8 *t*	2′, 18′	1, 3, 2′, 3′
2′	5.24 *t* (7.0)	122.4 *d*	1′, 18′	1′, 4′, 18′
3′		137.3 *s*		
4′a	2.09 *m*	39.3[b] *t*		3′, 5′, 18′
4′b	2.02 *m*			5′
5′	2.14 *m*	25.8 *t*		3′, 4′, 7′
6′	5.07 *m*	124.2 *d*	19′	4′, 5′, 8′, 19′
7′		134.7 *s*		
8′a	2.09 *m*	39.3[b] *t*		19′
8′b	2.02 *m*			7′, 10′
9′	2.16 *m*	26.0 *t*	10′	10′, 11′
10′	5.50 *t* (7.5)	131.1 *d*	9′	8′, 9′, 12′, 20′
11′		138.3 *s*		
12′	4.16 *dd* (5.5, 8.0)	77.1 *d*	13′a	10′, 11′, 13′, 14′, 20′
13′a	2.44 *m*	35.0 *t*	12′, 13′b	11′, 12′, 14′, 15′
13′b	2.24 *m*		13′a	12′, 15′
14′	5.09 *m*	119.8 *d*	16′, 17′	13′, 16′, 17′
15′		135.4 *s*		
16′	1.65 *s*	18.0 *q*	14′	14′, 15′, 17′
17′	1.73 *s*	25.9 *q*	14′	14′, 15′, 16′
18′	1.75 *s*	16.0 *q*	1′, 2′	2′, 3′, 4′
19′	1.58 *s*	16.1[b] *q*	6′	6′, 7′, 8′
20′	4.27 *d* (1.5)	58.4 *t*		10′, 11′, 12′
1′-OH	ND			
4′-OH	ND			
12′-OH	ND			
20′-OH	ND			

ND = Not Detected.
[a] Carbon assignments based on HSQCAD and DEPT experiments.
[b] Overlapped signals.

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignment of fallaquinone (**9**) in $CDCl_3$.

Position	δ_H (*J* in Hz)	δc[a], mult	gCOSY	gHMBC
1		188.2[c] *s*		
2		148.7 *s*		
3	6.45 *bs*	132.5 *d*	1′	1, 5, 1′
4		188.4[c] *s*		
5	6.54 *bs*	133.3 *d*	7	3, 4, 7
6		146.1 *s*		
7	2.05[b] *s*	16.3 *q*	5	1, 5, 6
1′	3.12 *d* (6.5)	27.9 *t*	2′, 3, 18′	1, 2, 3, 2′, 3′
2′	5.14 *m*	118.4 *d*	1′	
3′		140.2 *s*		
4′	2.04[b] *m*	39.7[b] *t*	5′	2′, 3′, 5′, 6′, 18′
5′	2.12 *m*	26.6 *t*	4′	4′, 6′, 7′
6′	5.10 *m*	124.6 *d*	19′	4′, 5′, 8′, 19′
7′		134.8 *s*		
8′	2.05[b] *m*	39.7[b] *t*	9′, 19′	6′, 7′, 9′
9′	2.20 *m*	26.1[c] *t*	8′, 10′	10′, 11′
10′	5.51 *t* (7.5)	130.7*d*	9′	8′, 12′, 20′
11′		138.7 *s*		
12′	4.16 *dd* (5.5)	77.1 *d*	13′a, 13′b	10′, 11′, 14′, 20′
13′a	2.25 *m*	35.2 *t*	12′, 14′	12′, 14′, 15′
13′b	2.43 *m*		12′	
14′	5.09 *m*	120.2 *d*	13′a, 17′	13′, 17′, 16′
15′		135.6 *s*		
16′	1.64 *s*	18.0 *q*		14′, 15′, 17′
17′	1.72 *s*	26.2[c] *q*	14′	14′, 15′, 16′
18′	1.62 *s*	16.4[b] *q*	1′	2′, 3′, 4′
19′	1.59 *s*	16.4[b] *q*	6′, 8′	6′, 7′, 8′
20′	4.26 *d* (2.5)	58.8 *t*		10′, 11′, 12′
12′-OH	ND			
20′-OH	ND			

ND = Not Detected.
[a] Carbon assignments based on HSQCAD and DEPT experiments.
[b] Overlapped signals.
[c] Interchangeable signals.

sion of **8** to its oxidized analogue fallaquinone (**9**), which is typical behaviour for hydroquinones on exposure to air (Mori et al., 2005). Analysis of the NMR spectra of **8** (Table 1) revealed chemical signals typical of a meroditerpenoid with diagnostic ^{1}H and ^{13}C NMR shifts of a terpene chain. The ^{1}H NMR spectrum showed the presence of an AB system (δ_H 6.51 and 6.48, J = 3.0 Hz), which was assigned to two *meta*-coupled aromatic protons with ^{13}C NMR chemical shifts of 114.1 and 115.6 ppm, respectively. The characteristic chemical shift for the methylene doublet (δ_H 3.28, δ_C 29.8) immediately suggested a hydroquinone to terpene chain linkage. The ^{1}H NMR spectrum also revealed vicinal coupling of the 1′ methylene (δ_H 3.28, *d*, *J*=7.0 Hz, δ_C 29.8) with a vinyl proton (δ_H 5.24, *t*, *J*=7.0 Hz, δ_C 122.4) together with resonances from deshielded methines (δ_H 5.07, 5.09, 5.25), five allylic methylenes (complex multiplet between δ_H 2.02 and 2.44) and four vinyl methyls (δ_H 1.65, 1.73, 1.75, 1.65) (Kusumi et al., 1979; Gerwick and Fenical, 1981). It quickly became apparent that the unique aspects on the terpene chain were the presence of a deshielded methine (δ_H 5.50, δ_C 131.1) at C10′, a deshielded methylene (δ_H 4.27, δ_C 58.4) at C20′ and the secondary alcohol methine (δ_H 4.16, δ_C 77.1) at C12. HMBC NMR correlations were observed from the methine and methylene moieties to the secondary alcohol methine (δ_H 4.16, δ_C 77.1), which confirmed the unique fragment at positions 10′, 11′, 12′ and 20′ of the linear terpene chain moiety. HMBC correlations were observed from this methylene doublet (δ_H 3.28, δ_C 29.8) to the aromatic methines [(δ_H 6.51, δ_C 115.6) and (δ_H 6.49, δ_C 114.1)], the benzohydroquinone moiety (δ_C 149.3 and δ_H 146.0) and a deshielded aromatic methyl (δ_H δ 2.14, δ_C 25.7), thereby linking the linear terpene to the hydroquinone moiety. HMBC correlations from the methylene [(δ_H 4.27, δ_C 58.4) to C10′, C11′ and C12′] allowed the primary alcohol to positioned at C11′.

Fallaquinone (**9**) was immediately recognised to be the quinone analogue of fallahydroquinone (**8**) on the basis of the similarity of the ^{1}H NMR spectrum and ^{13}C NMR chemical shifts (Table 2) to **8**. In particular the presence of the methylene doublet (δ_H 3.12, δ_C 27.9) suggested a benzoquinone to terpene chain linkage. Fallaquinone (**9**) was isolated as a pale yellow oil for which high resolution ESIMS established the molecular formula as $C_{27}H_{38}O_4$ (HRESIMS *m/z* 425.2691 $[M-H]^-$ calcd for $C_{27}H_{37}O_4$, 425.2692), possessing 9° of unsaturation. The IR spectrum of fallaquinone (**9**) was similar to fallahydroquinone (**8**) displaying the presence of hydroxy (3391 cm^{-1}), ketone (1653 cm^{-1}) and olefinic moieties (1463 cm^{-1}). The HMBC NMR spectrum further supported the presence of the benzoquinone moiety. Key HMBC correlations were observed from the aromatic methane protons at positions 3 (δ_H 6.45, *bs*) and 5 (δ_H 6.54, *bs*) to the carbonyl carbons (δ_C 188.4 and δ_C 188.2), which are characteristic signals of the benzoquinone moiety (Iwashima et al., 2005). As already mentioned fallaquinone (**9**) is formed as a result of the oxidation of **8** on exposure to air and is therefore more than likely an artefact (Mori et al., 2005).

Fallachromenoic acid (**10**) was isolated as the major compound co-occuring in a mixture with the minor compound sargaquinoic acid (**2**) in a 3:1 ratio, as was evident in the HRESIMS and NMR spectra. The high resolution ESIMS for fallachromenoic acid (**10**) established the molecular formula as $C_{27}H_{35}ClO_4$ (457.2149 $[M-H]^-$, calcd for $C_{27}H_{34}{}^{35}ClO_4$, 457.2145), possessing 10° of unsaturation and the presence of the chlorine was supported by the 3:1 isotopic ratio in the mass spectrum. The IR spectrum supported the presence of hydroxy (3400 cm^{-1}), ketone (1689 cm^{-1}) and olefinic moieties (1454 and 1590 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **10** displays chemical shifts typical of a chromene moiety attached to terpene

chain possessing a carboxylic group (Kusumi et al., 1979; Seo et al., 2007). The NMR data for **10** was compared to the literature data of structurally related sargachromenol (**11**). The distinctive feature that differed between compounds **10** and **11** was the presence of the deshielded methine carbon at position 11′ (δ_H 4.38, δ_C 66.2 ppm), indicative of the presence of a chlorine substituent and the linear chain terminal olefinic bond at position 13′ (δ_H 4.89 and 5.01, δ_C 66.2) in fallachromenoic acid (**10**). The placement of the chlorine moiety at position 11′ was supported by HMBC correlations to the deshielded methine at position 11′ (δ_C 66.2), at which the chlorine is attached, from positions 9′ (δ_H 2.26, *m*), 10′ (δ_H 2.00, *t*), 14′ (δ_H 1.81, *s*) and the double bond methylene proton at position 13′a (δ_H 4.89, *m*). The position of the linear chain terminal at 13′ was evident by the HMBC correlation from position 13′a (δ_H 4.89, *m*) to 11′ (δ_C 66.2) and 14′ (δ_C 17.2), and 13′b (δ_H 5.01, *m*) to 14′ (δ_C 17.2) (see Table 3). The structural analogue of **10**, sargachromenol (**11**) was also isolated and identified on the basis of 1D and 2D NMR data as well as by comparison to the literature and found to be identical in all respects (Kusumi et al., 1979; Seo et al., 2007). As a result of this study, additional structural characterisation data of **11** has now been reported for this compound.

Stereochemical assignment of the double bonds of the meroditerpenoids **8**–**10** was made on the basis of the position of the upfield vinyl methyl resonances in the ^{13}C NMR spectrum (δ_C 16.0–18.0) for these compounds (Cimino et al., 1972; Ishitsuka et al., 1979; Kasparek, 1980; Wehrli and Nishida, 1979). The *E* configuration of the double bond at C10′–C11′ in 8 and 9 was determined by comparison of the chemical shifts of the olefinic proton at C10′ and the C9′ methylene protons with those reported for *E*- and *Z*-2-methyl-2-pentenoic acids (Kusumi et al., 1979; Chan et al., 1968). Owing to the instability and rapid decomposition of the meroditerpenoids isolated, attempts to secure the relative or absolute configurations for the new compounds **8**, **9** and **10** could not be carried out.

Metabolites produced by the *Sargassum* sp. have been reported to display a range of biological activities. Plastoquinones isolated from the brown alga *Sargassum micracanthum* have been shown to contribute towards the diversity and selectivity in the bioactive properties of this genus. (Mori et al., 2005; Komai et al., 2006; Iwashima et al., 2005). Compound **4**, isolated from *S. micracanthum*, displayed significant antioxidant activity and subsequent investigation of various analogues of this compound, it was concluded that the activity was attributable to the hydroquinone moiety (Mori et al., 2005). Compound **4** also displayed potent cytotoxic activity against Colon 26-L5 cells, however the structure activity relationship and pharmacophore remains unknown (Mori et al., 2005). Compounds **5**–**7** isolated from *S. micracanthum* have also been evaluated in a number of assays. It was found that compound **5** possessed the strongest antioxidant activity, which was again, ascribed to the presence of the hydroquinone and phenol moieties (Iwashima et al., 2005). In addition it was found that compounds **6** and **7** displayed potent antiviral activity against human cytomegalovirus (HCMV), whereas compound **5** was virtually inactive in this case (Iwashima et al., 2005). Further investigation of the biological activities of **5**–**7** suggested the possibility that these compounds may also be future candidates for antiulcer effects and prevention of bone diseases such as osteoporosis (Mori et al., 2005; Komai et al., 2006).

In the evaluation of the bioactivity of the isolated meroterpenoids, sargaquinoic acid (**2**) and sargahydroquinoic acid (**3**) were found to display moderate antitumour activity (IC_{50} of 17 and 14 μM, respectively, when tested at 1 mg/mL in the P388 assay). Sargaquinone (**1**), fallahydroquinone (**8**), fallaquinone (**9**), fallachromenoic acid (**10**) and sargachromenol (**11**) displayed lower antitumour activities (IC_{50} of 32 μM for **1** and >27–29 μM for **8**–**10** when tested at 1 mg/mL in the P388 assay). Sargaquinoic acid (**2**) and sargahydroquinoic acid (**3**) were evaluated for antimicrobial activity and displayed only weak activity against *Bacillus subtilis*.

Previously, sargaquinoic acid (**2**) and sargachromenol (**11**) were reported to be neuroactive substances that significantly promote neurite outgrowth and support the survival of neuronal cells (Tsang and Kamei, 2004; Tsang et al., 2005). Also, sargaquinoic acid (**2**) and sargachromenol (**11**) in combination with UVB, demonstrated apoptotic effects, suggesting their potential use as therapeutic agents against hyperproliferative diseases such as psoriasis (Hur et al., 2008).

As a result of this study of the marine brown alga *S. fallax*, the biological evaluation (antitumour and some antimicrobial activites) of the meroterpenoids **2**, **3**, **8**, **9**, **10** and **11** have provided further insight into the bioactivity of these secondary metabolites in relation to the previously reported bioactivities for the related structural analogues **1–7**. Every effort was made to evaluate the biological activity for the isolated meroditerpenoids as rapidly as possible. Given their instability it is possible that the actual activity could in fact be greater than that reported here. Nevertheless, the results are in general agreement with trends reported from previous meroditerpenoids studies.

Table 3
^1H (500 MHz) and ^{13}C (125 MHz) NMR assignment of fallachromenoic acid (**10**) in $CDCl_3$.

Position	δ_H (*J* in Hz)	δc, mult	gCOSY	gHMBC
2		78.2 *s*		
3	5.56 *d* (9.8)	130.8 *d*	4	2, 4a
4	6.24 *d* (9.8)	123.4 *d*	3	8a
4a		121.7 *s*		
5	6.35 *d* (2.7)	110.8 *d*	7	4, 7, 8a
6		145.3 *s*		
7	6.50 *d* (2.7)	118.0 *d*	5	5, 6
8		126.3 *s*		
8a		144.8 *s*		
1′	1.67 *s*	40.7 *t*	2′	2, 2′
2′	2.12 *m*	23.0 *t*	1′, 3′	1′, 3′, 4′
3′	5.13 *m*	125.2 *d*	2′	2′
4′		132.2 *s*		
5′	2.05 *m*	39.3 *t*	6′	6′, 16′
6′	2.56 *q* (7.5)	28.2 *t*	5′, 7′	4′, 5′, 7′, 8′
7′	5.89 *t* (7.6)	143.1 *d*	6′	15′
8′		134.8 *s*		
9′	2.26 *t* (7.4)	35.2 *t*	10′	7′, 8′, 10′, 11′, 15′
10′	2.00 *t* (7.4)	36.2 *t*	9′, 11′	9′, 11′, 12′
11′	4.38 *m*	66.2 *d*	10′	9′, 14′
12′		144.5 *s*		
13′a	4.89 *m*	114.5 *t*		11′, 14′
13′b	5.01 *m*		14′	14′
14′	1.81 *s*	17.2 *q*	13′b	11′, 12′, 13′
15′		170.1 *s*		
16′	1.57 *s*	16.0 *q*		3′, 4′, 5′
17′	1.35 *s*	26.1 *q*		2, 3, 1′
18′	2.13 *s*	15.6 *q*		7, 8, 8a
6-OH	ND			
15′-COOH	ND			

ND = Not Detected.

3. Experimental section

3.1. General experimental procedures

For details on the general experimental procedures please see Reddy and Urban (2008).

HRESIMS was carried out on 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 0.1 mL/min) in either the positive or negative ionization modes. In all cases the instruments were calibrated using the 'Agilent Tuning Mix' using purine as the reference compound and the Hewlett–

Packard standard HP0921. All analytical HPLC analyses for fraction analyses and method development 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 0–2 min 10% CH_3CN/H_2O; 14–24 min 75% CH_3CN/H_2O; 26–30 min 100% CH_3CN and 32–40 min 10% CH_3CN/H_2O or an isocratic method (either 80% CH_3CN/H_2O or 85% CH_3CN/H_2O) on a Phenomenex Prodigy ODS (3) C_{18} 100 Å 250 × 4.6 (5 μ) and on a Phenomenex Luna ODS (3) C_{18} 100 Å 250 × 4.6 (5 μ) column at a flow rate of 1.0 mL/min. Semi-preparative HPLC was carried out on a Dionex P680 solvent delivery system equipped with a PDA100 UV detector (operated using "Chromeleon" software) using an isocratic method (85% CH_3CN/H_2O) and a Phenomenex Prodigy ODS (3) 100Å C_{18} 250 × 10 (5 μ) column at a flow rate of 3.5 mL/min.

3.1.1. Biological evaluation and details of assays

Extracts of the alga were evaluated in a number of biological assays at 50 mg/mL including against a P388 Murine Leukaemia cell line (antitumour assay), against *Herpes simplex* and *Polio* viruses (antiviral assays) as well as against a number of bacteria and fungi (antimicrobial assays) at the University of Canterbury, Christchurch, New Zealand. Moderate antitumour activity was observed for the alga extract (IC_{50} of 6984 ng/mL). In addition, the extract displayed cytotoxic activity against the *Herpes simplex* virus and the *Polio* virus as well as moderate antimicrobial activity with a zone of inhibition detected against *B. subtilis*. No activity was observed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans, Trichophyton mentagrophytes* or *Cladosporium resinae*.

3.1.2. Antitumour assay (P388 Murine Leukaemia cell line)

For the antitumour assay a twofold dilution series of the crude extract as well as compounds **1**–**3** and **8**–**11** were incubated for 72 h with P388 (Murine Leukaemia) cells. The concentration of sample required to reduce the P388 cell growth by 50% (compared to control cells) was determined using the absorbance values obtained when the yellow dye MTT tetrazolium is reduced by healthy cells to the purple coloured MTT formazan and is expressed as an IC_{50} (ng/mL).

3.1.3. 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.

3.1.4. Antimicrobial assays

A standardized inoculum was prepared by transferring a loop of bacterial/fungal cells, from a freshly grown stock slant culture, into a 10 mL vial of sterile water. This was vortexed and compared to a 5% $BaCl_2$ in water standard to standardize the cell density. This gave a cell density of 10^8 colony-forming units per mL. Ten millilitre of the standardized inoculum was then added to 100 mL of Mueller Hinton or potato dextrose agar (at between 40–50 °C) and mixed by swirling, giving a final cell density of 10^7 colony-forming units per mL. Five millilitre of this was poured into sterile 85 mm petri dishes. The suspensions were allowed to cool and solidify on a level surface to give a 'lawn' of bacteria/fungi over the dish (for further information see www.clsi.org). The crude extract as well as compounds **2** and **3** were pipetted onto 6 millimeter 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 mm as the radius of inhibition for each bacteria/fungi. The six organisms were *Eschericha coli* (G−ve ATCC 25922), *B. subtilis* (G+ve ATCC 19659) and *P. aeruginosa* (G−ve ATCC27853) for the bacteria and *C. albicans* (ATCC 14053), *T. 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.

3.1.5. Marine alga material

The marine brown alga (*S. fallax*) was collected by SCUBA at a depth of 2–4 m on the 11th September, 2003 from Governor Reef near Indented head, 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 number 2003-22) is deposited at the School of Applied Sciences (Discipline of Applied Chemistry), RMIT University.

3.1.6. Extraction and Isolation

The alga (36.3 g, wet weight) was extracted with 3:1 MeOH/DCM (500 mL) and the crude extract was decanted and concentrated under reduced pressure and subsequently sequentially solvent partitioned into DCM, MeOH and water-soluble extracts, respectively. The DCM extract was subjected to a flash silica gel column (20% stepwise elution from petroleum spirits (60–80 °C) to DCM to EtOAc and finally to MeOH). The 100% EtOAc silica gel column fraction was subjected to gel permeation chromatography (Sephadex LH-20 using 100% MeOH) followed by reversed phase HPLC (80% CH_3CN/H_2O) resulting in the isolation of fallahydroquinone (**8**) (10 mg, 0.13%). The 40:60 DCM/EtOAc silica gel column fraction was subjected to gel permeation chromatography (Sephadex LH-20 using 100% MeOH) followed by reversed phase HPLC (85% CH_3CN/H_2O) to yield sargaquinoic acid (**2**) (8 mg, 0.1%), sargahydroquinoic acid (**3**) (8 mg, 0.1%), fallaquinone (**9**) (5.0 mg, 0.06%) and sargachromenol (**11**) (10 mg, 0.13%). The remaining DCM partition was separately purified by reversed phase HPLC to yield sargaquinone (**1**) (5 mg, 0.06%) and fallachromenoic acid (**10**) (5 mg, 0.06%), wherein the percentage yields are reported on the basis of the dry mass of the alga extracted.

2-methyl-6-((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohexa-2,5-diene-1,4-dione sargaquinone (**1**); isolated as a pale yellow unstable oil; UV λ_{max}^{EtOH} (log ε) 255 nm (4.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3392, 2926, 2854, 1715, 1653, 1455, 1378, 1292; ^1H and ^{13}C NMR data identical to that previously reported (Amico et al., 1985).

(2*Z*,6*E*,10*E*)-6,10-dimethyl-12-(5-methyl-3,6-dioxocyclohexa-1,4-dienyl)-2-(4-methylpent-3-enyl)dodeca-2,6,10-trienoic acid sargaquinoic acid (**2**); isolated as a pale yellow unstable oil; UV λ_{max}^{EtOH} (log ε) 252 nm (4.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3337, 2925, 2854, 1686, 1655, 1439, 1377, 1293; ^1H NMR (500 MHz, $CDCl_3$): δ 6.54 (1H, *dq*, *J* = 1.5, 2.5 Hz, H-5), δ 6.46 (1H, *dq*, *J* = 1.5, 2.5 Hz, H-3), 5.95 (1H, *t*, *J* = 7.0 Hz, H-10′), 5.14 (1H, *m*, H-2′), 5.11 (1H, *m*, H-6′), 5.09 (1H, *m*, H-14′), 3.12 (2H, *d*, *J* = 7.0 Hz, H-1′), 2.58 (2H, *d*, *J* = 7.0 Hz, H-9′), 2.26 (2H, *t*, *J* = 7.0 Hz, H-12′), 2.11 (2H, *m*, H-13′), 2.09 (2H, *m*, H-5′), 2.06 (4H, *m*, H-4′ and H-8′[1]), δ 2.05 (3H, *s*, H-7), 1.67 (3H, *s*, H-17′), 1.61 (3H, *s*, H-18′), 1.59 (3H, *s*, H-19′), 1.58 (3H, *s*, H-16′), exchangeable protons not observed; ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.0 *s*, C-1), 187.9 (*s*, C-4), 171.8 (*s*, C-20′); 148.5 (*s*, C-2), 145.9 (*s*, C-6), 144.4 (*d*, C-10′), 139.8 (*s*, C-3′), 134.6 (*s*, C-7′), 133.1 (*d*, C-5), 132.2 (*d*, C-3), 132.1 (*s*, C-15′), 130.9 (*s*, C-11′),

[1] Overlapped signals.

124.4 (*d*, C-6′), 123.5 (*d*, C-14′), 117.9 (*d*, C-2′), 39.5 (*t*, C-4′), 39.0 (*t*, C-8′), 34.6 (*t*, C-12′), 28.2 (*t*, C-9′), 27.5 (*t*, C-1′), 27.8 (*t*, C-13′), 26.3 (*t*, C-5′), 25.6 (*q*, C-17′), 17.7 (*q*, C-16′), 16.1 (*q*, C-7), 16.0 (*q*, C-18′), 15.9 (*q*, C-19′); ESIMS (negative ion mode) *m/z* 423 $[M-H]^-$.

(2*Z*,6*E*,10*E*)-12-(2,5-dihydroxy-3-methylphenyl)-6,10-dimethyl-2-(4-methylpent-3-enyl)dodeca-2,6,10-trienoic acid sargahydroquinoic acid (**3**); isolated as a pale yellow unstable oil; λ_{max}^{EtOH} (log ε) 251 (5.05); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3418, 2922, 2854, 1686, 1670, 1654, 1614, 1439, 1377, 1294, 1260, 1194; ^1H NMR (500 MHz, $CDCl_3$): δ 6.51 (1H, *d*, *J* = 3.0 Hz, H-5), δ 6.48 (1H, *d*, *J* = 3.0 Hz, H-3), 5.95 (1H, *t*, *J* = 7.0 Hz, H-10′), 5.26 (1H, *t*, *J* = 7.0 Hz, H-2′), 5.11 (1H, *t*, *J* = 6.5 Hz, H-6′), 5.09 (1H, *t*, *J* = 6.0 Hz, H-14′), 3.28 (2H, *d*, *J* = 7.0 Hz, H-1′), 2.57 (2H, *q*, *J* = 7.5 Hz, H-9′), 2.26 (2H, *t*, *J* = 7.0 Hz, H-12′), 2.17 (3H, *s*, H-7), 2.13 (4H, *m*, H-5′ and H-13′[1]), 2.07 (4H, *m*, H-4′ and H-8′[1]), 1.74 (3H, *s*, H-18′), 1.67 (3H, *s*, H-17′), 1.59 (3H, *s*, H-16′), 1.58 (3H, *s*, H-19′), exchangeable protons not observed; ^{13}C NMR (125 MHz, $CDCl_3$) 171.7 (*s*, C-20′), 149.0 (*s*, C-4), δ 146.2 (*s*, C-1), 144.5 (*d*, C-10′), 138.0 (*s*, C-3′), 134.8 (*s*, C-7′), 132.2 (*s*, C-15′), 130.9 (*s*, C-11′), 127.6 (*s*, C-2), 125.5 (*s*, C-6), 124.2 (*d*, C-6′), 123.5 (*d*, C-14′), 121.8 (*d*, C-2′), 115.5 (*d*, C-5), 114.0 (*d*, C-3), 39.5 (*t*, C-4′), 39.1 (*t*, C-8′), 34.6 (*t*, C-12′), 29.9 (*t*, C-1′), 28.3 (*t*, C-13′), 27.8 (*t*, C-9′), 26.0 (*t*, C-5′), 25.6 (*q*, C-17′), 17.7 (*q*, C-16′), 16.2 (*q*, C-18′), 16.1 (*q*, C-7), 16.0 (*q*, C-19′). In the time it took to acquire the mass spectrum for sargahydroquinoic acid (**3**) it had converted to sargaquinoic acid (**2**).

2-((2*E*,6*E*,10*E*)-12-hydroxy-11-(hydroxymethyl)-3,7,15-trimethylhexadeca-2,6,10,14-tetraenyl)-6-methylbenzene-1,4-diol fallahydroquinone (**8**), isolated as a pale yellow unstable oil; $[\alpha]_D^{25}$ + 54.9° (c 0.08, $CHCl_3$); UV λ_{max}^{EtOH} (log ε) 256 nm (4.0); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3368, 2963, 2920, 2859, 1652, 1614, 1461, 1441, 1377, 1315, 1196, 1144; ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS (positive ion mode) *m/z* 451 $[M+Na]^+$ and 467 $[M+K]^+$; ESIMS (negative ion mode) *m/z* 427 $[M-H]^-$; HRESIMS *m/z* 451.2812 $[M+Na]^+$; calcd for $C_{27}H_{40}O_4Na$, 451.2824.

2-((2*E*,6*E*,10*Z*)-12-hydroxy-3,7-dimethyl-11-(4-methyl-3-enyl-pentan-1-ol)dodeca-2,6,10-trienyl)-6-methylcyclohexa-2,5-diene-1,4-dione fallaquinone (**9**), isolated as a pale yellow unstable oil; $[\alpha]_D^{25}$ – 12.5° (c 0.08, $CHCl_3$); UV λ_{max}^{EtOH} (log ε) 255 nm (3.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3390, 2951, 1667, 1589, 1463, 1377; ^1H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS (negative ion mode) *m/z* 425.3 $[M-H]^-$; 461.3 $[M+Cl]^-$; HRESIMS *m/z* 425.2691 $[M-H]^-$; calcd for $C_{27}H_{37}O_4$, 425.2692.

(2Z,6E)-2-(3-chloro-4-methylpent-4-enyl)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methylnona-2,6-dienoic acid fallachromenoic acid (**10**); isolated as a pale yellow unstable oil; $[\alpha]_D^{25}$ – 73.8° (c 0.03, $CHCl_3$); UV λ_{max}^{EtOH} (log ε) 340 nm (3.0); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3401, 2925, 2853, 1689, 1588, 1455, 1348, 1377; ^1H and ^{13}C NMR spectroscopic data, see Table 3; ESIMS (negative ion mode) *m/z* 457.0 $[M-H]^-$; HRESIMS *m/z* 457.2149 $[M-H]^+$; calcd for $C_{27}H_{34}{}^{35}ClO_4$, 457.2145.

(2*Z*,6*E*)-9-(6-hydroxy-2,8-dimethyl-2H-chromen-2-yl)-6-methyl-2-(4-methylpent-3-enyl)nona-2,6-dienoic acid sargachromenol (**11**); isolated as a pale yellow unstable oil; $[\alpha]_D^{25}$ – 23.7° (c 0.07, $CHCl_3$); UV λ_{max}^{EtOH} (log ε) 330 nm (3.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3401, 2964, 2924, 2853, 1683, 1652, 1462, 1377, 1312, 1256, 1216; ^1H and ^{13}C NMR identical to that previously reported (Kusumi et al., 1979; Seo et al., 2007); ESIMS (negative ion mode) *m/z* 423 $[M-H]^-$.

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