

## Dictyosphaerin: A Novel Bicyclic Lipid from a Southern Australian Marine Green Alga, *Dictyosphaeria sericea*

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The novel bicyclic lipid, dictyosphaerin (**1**), has been isolated from the southern Australian marine green alga *Dictyosphaeria sericea*. The molecular structure for **1** was secured by chemical derivatization and detailed spectroscopic analysis.

Historically, the natural product chemistry of marine Chlorophyta has featured terpenoid metabolites incorporating the unique diacetoxybutadiene functionality.<sup>1</sup> Furthermore, many of these metabolites have been shown to possess potent antifeedant properties, presumably through hydrolysis of the diacetoxybutadiene functionality, to yield the biologically reactive 1,4-dialdehyde moiety reminiscent of the known terpenoid antifeedant polygodial.<sup>2</sup> Despite an impressive biodiversity, it is noteworthy that very little has been published on the chemistry of Australian marine Chlorophyta, which consists entirely of a limited selection of terpenoids from collections of the cosmopolitan genus *Caulerpa*.<sup>3–6</sup> Other Australian Chlorophyta have either not returned novel secondary metabolites or, as is more likely the case, have not been subjected to chemical analysis. As a result of our ongoing investigations into the chemistry of southern Australian marine organisms we take this opportunity to describe a novel lipid from the indigenous Australian green alga *Dictyosphaeria sericea*.

*D. sericea* is indigenous to southern Australian waters and represents the only temperate species of an otherwise tropical genus. The alga can be found as small (1–3 cm) disk-like structures attached to the leading vertical and underhanging edges of intertidal rock platforms, particularly in areas of high wave activity. Dictyosphaerin (**1**) is the first novel lipid to be reported from an Australian marine green alga other than a *Caulerpa* species, and this is the first account of a natural product from the genus *Dictyosphaeria*.

Specimens of *D. sericea* were obtained from locations along the southern coast of Victoria, including Point Lonsdale, Flinders, Cape Schank, Warrnambool, and Point Impossible. Specimens from Cape Schank and Point Lonsdale provided the bulk of the extractable material, which consistently yielded a single, unstable novel lipid, identified as dictyosphaerin (**1**).

Dictyosphaerin (**1**) possessed a molecular formula (C<sub>22</sub>H<sub>34</sub>O<sub>3</sub>, Δ<sub>mmu</sub> +0.9) that required six double-bond equivalents (DBE). IR absorptions [2900 (br), 1710 cm<sup>-1</sup>] and a deshielded <sup>13</sup>C-NMR (CDCl<sub>3</sub>) resonance [179.1 (s) ppm] indicated a carboxylic acid functionality, confirmed when methylation with CH<sub>2</sub>N<sub>2</sub> yielded the corresponding methyl ester **2** (<sup>1</sup>H: δ 3.65).<sup>7</sup> A further IR absorption [3400 (br) cm<sup>-1</sup>] and the presence of an

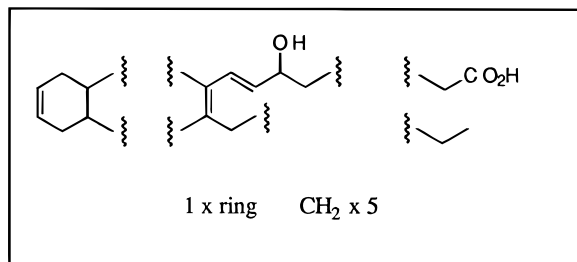


Figure 1. Structural fragments of dictyosphaerin (**1**).

oxygenated <sup>13</sup>C-NMR resonance [73.2 (d) ppm] supported the presence of a secondary alcohol. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) coupling between the oxymethine proton and protons for an *E* 1,2-disubstituted double bond (<sup>1</sup>H, δ 5.53, dd, *J* = 6.8, 14.3 Hz and δ 6.50, d, *J* = 15.0 Hz) confirmed the hydroxy substituent as being allylic. Acetylation of **2** to produce the acetate **3** (<sup>1</sup>H, δ 2.04) supported this conclusion, with the allylic oxymethine proton undergoing a characteristic downfield <sup>1</sup>H-NMR shift (δ 4.17 in **2** versus δ 5.30 in **3**).

The appearance of six "olefinic" carbon resonances (145.7 s, 133.8 s, 131.1 d, 128.4 d, 127.0 d, and 125.4 d ppm) suggested the presence of three double bonds, while a UV absorption (241 nm) indicated the presence of a conjugated diene. Also evident from the NMR evidence was the presence of an aliphatic primary methyl (<sup>1</sup>H, δ 0.87, t, *J* = 6.8 Hz and <sup>13</sup>C, 14.1, q). The remaining two DBEs required that **1** be bicyclic. An ion in the EIMS of **1** at *m/z* 274, consistent with the loss of a C<sub>4</sub>H<sub>6</sub> fragment from the base peak M<sup>+</sup> – H<sub>2</sub>O ion at *m/z* 328, was attributed to a retro-Diels-Alder fragmentation from a cyclohexene subunit. Consideration of the observations made above, in concert with 2D-NMR data, supported the structure fragments detailed in Figure 1. Careful analysis of the COSY and gHMBC data of the methyl ester **2** with addition of a Eu(fod)<sub>3</sub> shift reagent (Table 1) suggested assembly of the structure fragments as shown. Particularly instructive were the chemical shifts for the methine protons 11-H (δ 1.69) and 16-H (δ 2.30), indicating only the latter to be allylic.<sup>8</sup> Overlapping <sup>1</sup>H-NMR resonances prevented correlations from defining the complete structure for dictyosphaerin and its derivatives.

A sequence of hydrogenation followed by oxidation successfully transformed the methyl ester **2** into the saturated ketone **4**. Mass spectral analysis of **4** revealed fragmentations diagnostic for a McLafferty cleavage of the C-7 to C-8 bond (*m/z* 158, C<sub>8</sub>H<sub>14</sub>O<sub>3</sub>, Δ<sub>mmu</sub> +0.4), which, together with the structure fragments shown in Figure 1, unambiguously defined the complete struc-

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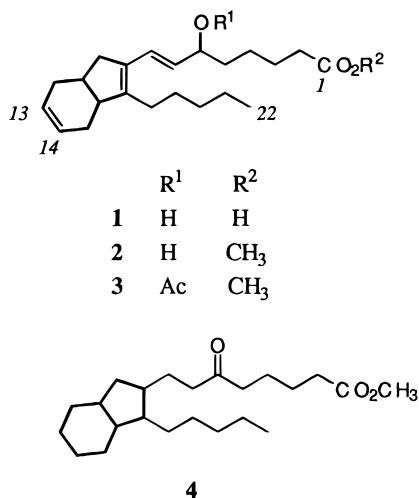
<sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, November 15, 1996.

**Table 1.** NMR Data [400 MHz, CDCl<sub>3</sub> + 0.03 mole equivalents of the shift reagent Eu(fod)<sub>3</sub>] for Dictyosphaerin Methyl Ester 2

carbon	ppm (m)	proton (m, J/Hz)	COSY	gHMBC
1	175.1 (s)			
2	34.2 (t)	2.44 (t, 7.5)	1.80	C-1, C-3
3	25.0 (t)	1.80 (m) <sup>b</sup>	2.44, 1.60	C-1, C-2, C-4
4	25.3 (t)	1.60 (m)	1.80, 1.70	C-2, C-3, C-5
5	37.2 (t)	1.70 (m) <sup>c</sup> 1.80 (m) <sup>b</sup>	1.80, 1.60, 4.42 1.70, 1.60, 4.42	C-4 C-4, C-7
6	73.8 (d)	4.42 (m)	1.70, 1.80, 5.70	C-8
7	131.2 (d)	5.70 (dd, 7.1, 15.5)	4.42, 6.62	C-5, C-6
8	125.5 (d)	6.62 (d, 15.6)	5.70	C-10
9	133.8 (s)			
10	37.0 (t)	2.02 (m) <sup>d</sup> 2.58 (dd, 7.1, 14.0)	2.58, 1.69 2.02, 1.69	C-9, C-11, C-12, C-16, C-17 C-9, C-11, C-16, C-17
11	42.4 (d)	1.69 (m) <sup>c</sup>	2.02, 2.58, 2.28–2.30	C-10, C-15, C-16
12 <sup>a</sup>	31.2 (t)	2.02 (m) <sup>d</sup> 2.28 (m) <sup>e</sup>	1.69, 2.28 2.02, 1.69, 5.75	C-11, C-13, C-14, C-16 C-11, C-13
13	128.4 (d)	5.75 (m) <sup>f</sup>	2.28–2.32	C-16
14	127.0 (d)	5.75 (m) <sup>f</sup>	2.28–2.32	C-16
15 <sup>a</sup>	29.8 (t)	1.90 (m) 2.32 (m) <sup>e</sup>	2.28–2.32 1.90, 5.75	C-16 C-13
16	49.4 (d)	2.30 (m) <sup>e</sup>	1.90	C-9, C-11, C-13, C-17
17	145.8 (s)			
18	26.5 (t)	2.08 (m) <sup>d</sup> 2.33 (m) <sup>e</sup>	1.30, 1.42, 2.33 1.30, 1.42, 2.08	C-17, C-19, C-20 C-9, C-17, C-19, C-20
19	28.4 (t)	1.30 (m) <sup>g</sup> 1.42 (m)	1.42, 2.08, 2.33 1.30, 2.08, 2.33	C-21, C-22 C-20
20	31.8 (t)	1.30 (m) <sup>g</sup>	1.42	C-21, C-22
21	22.5 (t)	1.30 (m) <sup>g</sup>	0.88	C-20, C-22
22	14.0 (q)	0.88 (t, 6.8)	1.30	C-21
OCOCH <sub>3</sub>	51.6 (q)	3.77 (s)		C-1

<sup>a</sup> Resonances may be interchanged. <sup>b,c,d,e,f,g</sup> Overlapping multiplets—assignments are supported by COSY and gHMBC data.

tures for **1** to **4** as shown. Efforts at securing either a relative (via NOE) or absolute (via the Mosher procedure) stereochemistry for dictyosphaerin (**1**) proved unsuccessful.



It is interesting to note that not all collections of the alga *D. sericea* contained dictyosphaerin (**1**), with at least one collection from Point Lonsdale containing a triglyceride analogue that decomposed during isolation. To the best of our knowledge the carbon skeleton assigned to dictyosphaerin (**1**) is unique. Our investigation of this temperate species of the genus *Dictyosphaeria* suggests that chemical examination of tropical species of the same genus would prove fruitful.

## Experimental Section

**General Experimental Procedures.** General experimental conditions have been reported elsewhere.<sup>9</sup>

**Collection, Extraction, and Isolation.** Collections of *Dictyosphaeria sericea* from either Point Lonsdale or

Cape Schank were packed in ice, transported back to the laboratory, steeped in EtOH–CH<sub>2</sub>Cl<sub>2</sub> (9:1), and stored at –20 °C. The crude concentrated extracts were typically partitioned into CH<sub>2</sub>Cl<sub>2</sub>-soluble and CH<sub>2</sub>Cl<sub>2</sub>-insoluble fractions, with the former being subjected to rapid silica filtration (20% stepwise gradient elution from petroleum spirits (bp 40–60 °C) to EtOAc) followed by normal-phase HPLC (Phenomenex 5 μ silica 10 × 250 mm column, eluent 30% EtOAc/petroleum spirits, 2 mL/min) to yield dictyosphaerin (**1**) (~0.3% of dry algal wt).

**Dictyosphaerin (1):** isolated as an unstable oil; [α]<sub>D</sub> –50° (c 1.0, CHCl<sub>3</sub>); UV (EtOH) λ max (ε) 203 (13 500), 241 (10 000), 248 (10 500) nm; IR (film) ν max 3400, 2900, 1710 cm<sup>-1</sup>; <sup>1</sup>H NMR\* (CDCl<sub>3</sub>, 400 MHz) δ 0.87 (t, J = 6.8 Hz, H<sub>3</sub>-22), 2.54 (dd, J = 7.3, 14.0 Hz, H-10a), 4.19 (m, H-6), 5.53 (dd, J = 6.8, 14.3 Hz, H-7), 5.75 (br t, J = 9.0 Hz, H-13 and H-14), 6.50 (d, J = 15.0 Hz, H-8); <sup>13</sup>C NMR\* (CDCl<sub>3</sub>, 100 MHz) δ 179.1 (br s, C-1), 145.7 (s, C-17), 133.8 (s, C-9), 131.1 (d, C-7), 128.4 (d, C-13)<sup>a</sup>, 127.0 (d, C-14)<sup>a</sup>, 125.4 (d, C-7), 73.2 (d, C-6), 49.4 (d, C-16), 42.4 (d, C-11), 37.0 (t, C-5)<sup>b</sup>, 36.9 (t, C-10)<sup>b</sup>, 34.0 (br t, C-2), 31.8 (t, C-20), 31.3 (t, C-12), 29.8 (t, C-15), 28.4 (t, C-19), 26.5 (t, C-18), 25.0 (t, C-4)<sup>c</sup>, 24.7 (t, C-3)<sup>c</sup>, 22.5 (t, C-21), 14.1 (q, C-22); <sup>a, b, c</sup>these resonances may be interchanged; \*shifts assigned by comparison to dictyosphaerin methyl ester + shift reagent (Table 1); EIMS (70 eV) m/z [M<sup>+</sup>] 346 (0.9), 328 (100), 274 (46), 257 (26), 202 (44), 148 (43), 91 (88); HREIMS m/z 346.2499 calcd for C<sub>22</sub>H<sub>34</sub>O<sub>3</sub> 346.2508, m/z 328.2408 calcd for C<sub>22</sub>H<sub>32</sub>O<sub>2</sub> 328.2402, m/z 274.1932 calcd for C<sub>18</sub>H<sub>26</sub>O<sub>2</sub> 274.1933.

**Dictyosphaerin Methyl Ester 2.** To a solution of dictyosphaerin (**1**) (50 mg) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added several drops of CH<sub>2</sub>N<sub>2</sub>, and the resulting mixture was allowed to stand at room temperature for 5 min. Evaporation of the solvent returned dictyosphaerin

methyl ester (**2**) in quantitative yield as a stable oil:  $[\alpha]_D -47^\circ$  (*c* 0.7,  $\text{CHCl}_3$ );  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.87 (t,  $J = 6.5$  Hz,  $\text{H}_3$ -22), 2.54 (dd,  $J = 7.2$ , 14.1 Hz, H-10a), 3.65 (s,  $\text{COCH}_3$ ), 4.17 (m, H-6), 5.53 (dd,  $J = 7.0$ , 15.0 Hz, H-7), 5.73 (br t,  $J = 4.5$  Hz, H-13 and H-14), 6.50 (d,  $J = 15.5$  Hz, H-8);  $^{13}\text{C NMR}^*$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  174.1 (br s, C-1), 145.7 (s, C-17), 133.7 (s, C-9), 131.1 (d, C-7), 128.4 (d, C-13)<sup>a</sup>, 127.0 (d, C-14)<sup>a</sup>, 125.4 (d, C-7), 73.2 (d, C-6), 51.5 (q,  $\text{COCH}_3$ ), 49.4 (d, C-16), 42.4 (d, C-11), 37.0 (t, C-5)<sup>b</sup>, 36.9 (t, C-10)<sup>b</sup>, 34.0 (br t, C-2), 31.8 (t, C-20), 31.3 (t, C-12), 29.8 (t, C-15), 28.3 (t, C-19), 26.5 (t, C-18), 25.1 (t, C-4)<sup>c</sup>, 24.8 (t, C-3)<sup>c</sup>, 22.5 (t, C-21), 14.0 (q, C-22); <sup>a</sup>, <sup>b</sup>, <sup>c</sup>these resonances may be interchanged; \*shifts assigned by comparison to dictyosphaerin methyl ester + shift reagent (Table 1); EIMS (70 eV)  $m/z$  [ $\text{M}^+$ ] 360 (2), 342 (100), 288 (23), 271 (11), 253 (10), 202 (22), 157 (10), 143 (24), 117 (22), 91 (28), 67 (17); HREIMS  $m/z$  360.2653 calcd for  $\text{C}_{23}\text{H}_{36}\text{O}_3$  360.2664.

**6-Acetoxydictyosphaerin Methyl Ester 3.** Dictyosphaerin methyl ester **2** (5 mg) was stirred overnight at room temperature in a 1:1 mixture of  $\text{Ac}_2\text{O}$  and pyridine (2 mL). Evaporation of the solvent returned 6-acetoxydictyosphaerin methyl ester **3** in quantitative yield as a stable oil;  $[\alpha]_D -5^\circ$  (*c* 0.2,  $\text{CHCl}_3$ );  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.87 (t,  $J = 7.5$  Hz,  $\text{H}_3$ -22), 2.04 (s,  $\text{OCOCH}_3$ ), 2.52 (dd,  $J = 7.1$ , 14.0 Hz, H-10a), 5.30 (br dd,  $J = 7.5$ , 14.5 Hz, H-6), 5.41 (dd,  $J = 7.8$ , 15.4 Hz, H-7), 5.73 (m, H-13 and H-14), 6.55 (d,  $J = 15.5$  Hz, H-8); \*shifts assigned by comparison to dictyosphaerin methyl ester + shift reagent (Table 1); EIMS (70 eV)  $m/z$  [ $\text{M}^+$ ] 402 (7), 360 (22), 342 (100), 288 (18), 245 (27), 201 (25), 171 (14), 143 (50), 117 (49), 91 (93), 79 (67), 54 (59); HREIMS  $m/z$  402.2782 calcd for  $\text{C}_{25}\text{H}_{38}\text{O}_4$  402.2770.

**Hydrogenation of Dictyosphaerin Methyl Ester (2).** A solution of dictyosphaerin methyl ester **2** (35 mg) in EtOAc (5 mL) in the presence of a catalytic amount of Pd on C (10%) was stirred under 1 atm of  $\text{H}_2$  for 24 h, after which the solution was filtered and the solvent removed under reduced pressure to yield a colorless oil (33 mg, 94%). The two isomers formed by hydrogenation were separated by reversed-phase HPLC (Phenomenex 5  $\mu$   $\text{C}_{18}$  10  $\times$  250 mm column, eluent 5%  $\text{H}_2\text{O}$ /MeOH, 2 mL/min) to yield hexahydrodictyosphaerin methyl ester isomer A (14 mg) as a stable oil;  $[\alpha]_D +11^\circ$  (*c* 0.2,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.88 (t,  $J = 7.5$  Hz,  $\text{H}_3$ -22), 2.34 (t,  $J = 7.4$ , 14.0 Hz,  $\text{H}_2$ -2), 3.58 (m, H-6), 3.67 (s,  $\text{COCH}_3$ );  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  174.2 (br s, C-1), 71.8 (d, C-6), 52.5 (q,  $\text{COCH}_3$ ), 51.5 (d), 51.4 (d), 44.6 (d), 43.0 (d), 37.1 (t), 36.7 (t), 36.6 (t), 34.3 (t), 34.0\* (t), 33.3 (t), 32.6 (t), 32.0 (t), 31.2 (d), 27.8 (t), 26.5\* (t), 25.2 (t), 24.9\* (t), 22.7 (t), 14.2 (q, C-22); \*one methylene carbon is obscured by one of these resonances; EIMS (70 eV)  $m/z$  [ $\text{M}^+ - \text{H}_2\text{O}$ ] 348 (13), 277 (50), 251 (6), 233 (3), 206 (11), 191 (100), 145 (51),

135 (38), 121 (46), 95 (21), 87 (56), 54 (13); HREIMS  $m/z$  348.3033 calcd for  $\text{C}_{23}\text{H}_{40}\text{O}_2$  348.3028; and hexahydrodictyosphaerin methyl ester isomer B (15 mg) as a stable oil;  $[\alpha]_D -43^\circ$  (*c* 0.7,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.88 (t,  $J = 7.5$  Hz,  $\text{H}_3$ -22), 2.34 (t,  $J = 7.4$ , 14.0 Hz,  $\text{H}_2$ -2), 3.58 (m, H-6), 3.67 (s,  $\text{COCH}_3$ );  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  174.2 (br s, C-1), 72.3 (d, C-6), 51.5 (q,  $\text{COCH}_3$ ), 50.1 (d), 47.5 (d), 45.8 (d), 38.6 (t), 38.3 (d), 36.9 (t), 36.3 (t), 34.0 (t), 32.5 (t), 32.4 (t), 31.1 (t), 28.6 (2t), 28.2 (t), 26.7 (t), 26.4 (t), 25.2 (t), 24.9 (t), 22.7 (t), 14.2 (q, C-22); EIMS (70 eV)  $m/z$  [ $\text{M}^+ - \text{H}_2\text{O}$ ] 348 (10), 336 (2), 277 (35), 263 (4), 233 (3), 206 (17), 191 (100), 145 (38), 135 (48), 116 (31), 95 (17), 87 (35), 56 (6); HREIMS  $m/z$  348.3030 calcd for  $\text{C}_{23}\text{H}_{40}\text{O}_2$  348.3028.

**Oxidation of Hexahydrodictyosphaerin Methyl Ester Isomer B.** A solution of hexahydrodictyosphaerin methyl ester isomer B (12 mg, 0.033 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was treated with pyridinium dichromate (24 mg, 0.066 mmol) and stirred for 24 h, after which the reaction mixture was filtered through a plug of silica (EtOAc as eluent) to yield 7,8,9,13,14,17-hexahydro-6-oxodictyosphaerin methyl ester **4** as a colorless stable oil (9 mg, 76%);  $[\alpha]_D -45^\circ$  (*c* 0.3,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.88 (t,  $J = 6.9$  Hz,  $\text{H}_3$ -22), 2.32 (t,  $J = 7.0$ ,  $\text{H}_2$ -2), 2.42 (t,  $J = 5.3$ ,  $\text{H}_2$ -5 and  $\text{H}_2$ -7), 3.66 (s,  $\text{COCH}_3$ );  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  211.3 (s, C-6), 173.9 (br s, C-1), 51.5 (q,  $\text{COCH}_3$ ), 49.9 (d), 47.5 (d), 45.8 (d), 42.3 (t), 41.6 (t), 38.1 (t), 37.6 (d), 33.8 (t), 32.4 (t), 32.3 (t), 31.0 (t), 28.6 (t), 28.4 (t), 26.6 (t), 26.4 (2t), 24.5 (t), 23.2 (t), 22.7 (t), 14.2 (q, C-22); EIMS (70 eV)  $m/z$  [ $\text{M}^+$ ] 364 (46), 344 (15), 314 (13), 204 (13), 171 (11), 158 (67), 149 (3), 135 (34), 126 (43), 111 (18), 95 (42), 84 (100), 53 (92); HREIMS  $m/z$  364.2964 calcd for  $\text{C}_{23}\text{H}_{40}\text{O}_3$  364.2977,  $m/z$  158.0939 calcd for  $\text{C}_8\text{H}_{14}\text{O}_3$  158.0943.

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- Methylation also had the effect of stabilizing dictyosphaerin, which even in the dark at  $< 0^\circ\text{C}$  underwent complete decomposition in less than 2 weeks.
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