An Antiproliferative Bis-prenylated Quinone from the New Zealand Brown Alga *Perithalia capillaris*

Catherine E. Sansom,[†] Lesley Larsen,[†] Nigel B. Perry,[*,†] Michael V. Berridge,[‡] Elizabeth W. Chia,[‡] Jacquie L. Harper,[‡] and Victoria L. Webb[⊥]

TerraMarine Pharmaceuticals, New Zealand Institute for Crop & Food Research Ltd, Chemistry Department, University of Otago, P.O. Box 56, Dunedin, New Zealand, Malaghan Institute of Medical Research, P.O. Box 7060, Wellington South, New Zealand, and National Institute of Water & Atmospheric Research (NIWA) Ltd, P.O. Box 14-901, Kilbirnie, Wellington, New Zealand

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Bioactivity-directed isolation work on the endemic New Zealand brown alga *Perithalia capillaris*, seeking anti-inflammatory compounds, led to a new bis-prenylated quinone (**4**). This compound inhibited superoxide production by human neutrophils *in vitro* (IC_{50} 2.1 μM), but was more potent at inhibiting proliferation of HL60 cells (IC_{50} 0.34 μM). Two related bis-prenylated phenols were also isolated, one known (**2**) and one new (**5**), with weaker biological activities. This report extends the examples of bis-prenylated phenols as chemotaxonomic markers for brown algae of the order Sporochnales.

Screening for new classes of anti-inflammatory natural products that inhibit superoxide production by human neutrophils[1–3] led to an active extract of the New Zealand endemic brown alga *Perithalia capillaris* J. Agardh (family Sporochnaceae, order Sporochnales).[4,5] *P. capillaris* is a relatively large seaweed, up to 80 cm high, which grows on subtidal rocks around the warmer northern coasts of New Zealand.[4]

Terpenes and polyphenolics are the predominant metabolite classes found in brown algae.[6] Fenical has proposed that brown algae in the order Sporochnales are chemically unique in their production of phenols with either multiple isoprenoid or monoterpenoid substituents.[7] The only other *Perithalia* species, *P. caudata* from Australian waters,[5] has yielded bis-prenylated phenols **1**−**3**.[8–10] Compound **1** has also been reported from *Encyothalia cliftonii* (Sporochnales) as a deterrent to herbivore feeding,[7] and **2** was isolated from *Sporochnus pedunculatus* (Sporochnales) as an antimicrobial compound.[11] *P. caudata* also yielded farnesylated *p*-hydroxybenzoic acid[10] and a fatty-acid-derived pheromone.[12] We now report on the chemistry of the New Zealand species *P. capillaris* for the first time, which contains known phenol **2** plus new compounds **4** and **5**.

HPLC and TLC analyses of the anti-inflammatory[13] extract of *P. capillaris* showed mostly low-polarity compounds, which were separated by column chromatography over silica gel. The fraction most active in the anti-inflammatory assay contained predominantly one compound, **4**, with the formula $C_{16}H_{20}O_2$ by HREIMS. 2D NMR analyses (Supporting Information) showed that two prenyl groups were present. These were 1,1-dimethylprop-2-enyl and 3,3-dimethylprop-2-enyl, with ^{1}H and ^{13}C NMR signals (Table 1) very similar to those of compound **2** (see Blackman et al.[8] and Supporting Information).

The remaining portion of compound **4**, $C_6H_2O_2$, was shown to be a *para*-quinone by the carbonyl signals at 188.5 and 187.6 ppm (Table 1).[14] The two quinone proton signals were not detectably coupled to one another (Table 1), so the prenyl groups were either 2,5 or 2,6. The proposed structure of **4** as 5-(1,1-dimethylprop-2-enyl)-2-(3-methylbut-2-enyl)cyclohexa-2,5-diene-1,4-dione was based on the key 2D NMR correlations shown in Figure 1. Structure **4** was supported by similar quinone NMR signals shown by 2-geranyl-5-methyl *p*-benzoquinone (from a soft coral).[15] Structure **4** has not been reported previously from any source, and no 2,5-substituted quinones have been reported from brown algae (2,6-substituted quinones have been reported from other brown algae, e.g., *Cystoseira crinita*[16]).

Table 1. NMR Spectroscopic Data ($CDCl_3$) for Compounds **4** and **5**

	compound **4**		compound **5**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	188.5		146.4	
2	146.9		120.6	
3	134.1	6.40, t (2.0)	115.0	6.49, s
4	187.6		148.3	
5	154.1		132.9	
6	132.3	6.59, s	113.9	6.71, br s
1′	40.4		40.4	
2′	145.2	6.06, dd (17.0, 11.0)	147.6	6.15, dd (18.0, 11.0)
3′	112.7	5.02, br dd (11.0)	113.4	5.31, dd (18.0, 1.0)
		4.97, br dd (17.0)		5.26, dd (11.0, 1.0)
4′ + 5′	26.8	1.35, s	27.8	1.42, s
1″	26.8	3.06, br d (7.5)	121.7	6.24, d (10.0)
2″	118.0	5.11, tt (7.5, 1.5)	131.1	5.58, d (10.0)
3″	136.1		75.8	
4″	25.7	1.73, br s	26.9	1.40, s
5″	17.7	1.61, br s	26.9	1.40, s
4-OH				5.43, s

Another fraction from the first silica gel column contained compounds similar to **4** by ^{1}H NMR spectroscopy. This fraction was further purified by silica gel chromatography to give more of quinone **4**, plus compounds **2** and **5**. The known phenol **2** was

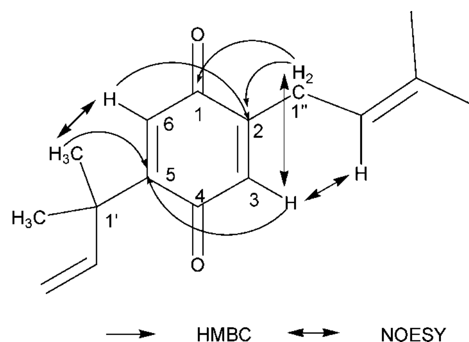

Figure 1. Key 2D NMR correlations for compound **4**.

* To whom correspondence should be addressed. E-mail: perryn@crop.cri.nz. Fax: +64-3-4798543.
[†] Crop & Food Research.
[‡] Malaghan Institute of Medical Research.
[⊥] NIWA.

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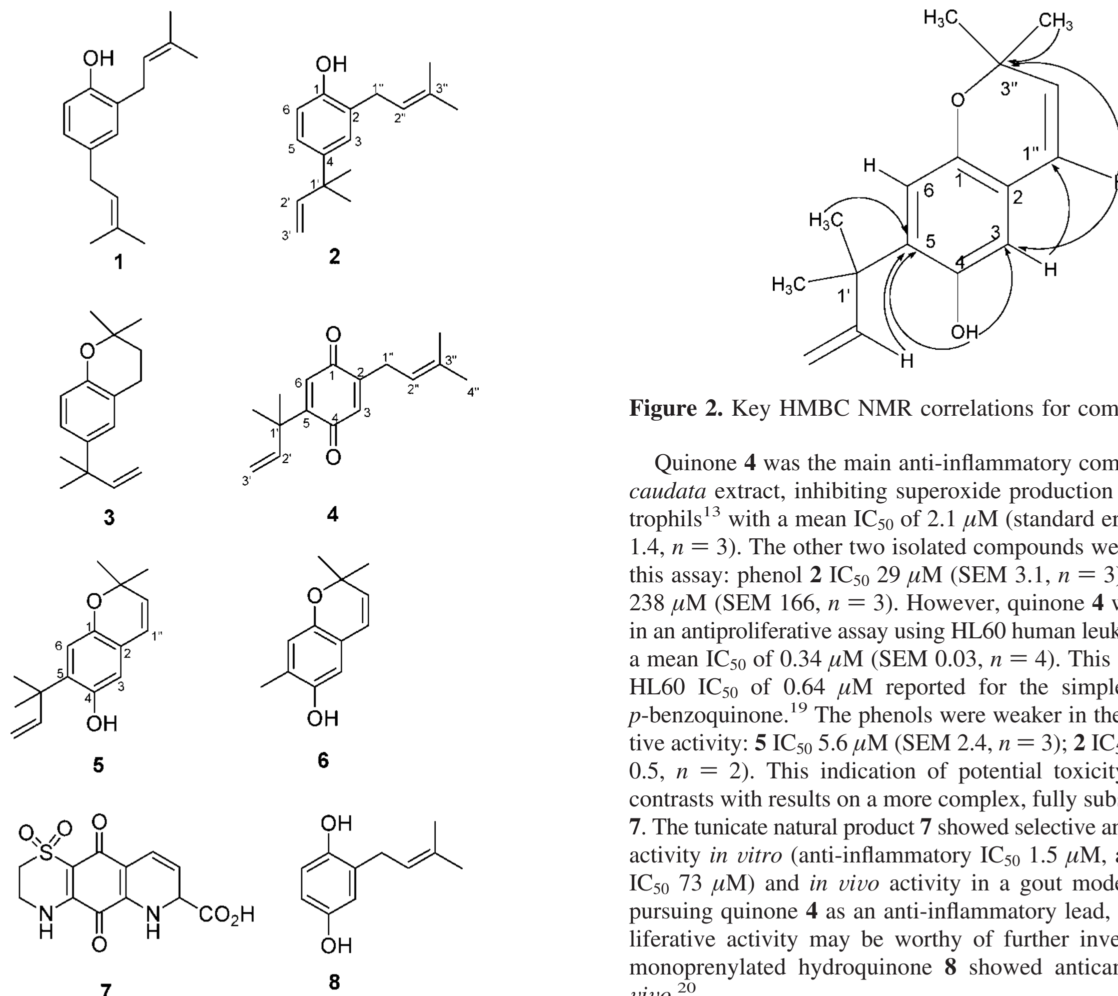

Figure 2. Key HMBC NMR correlations for compound **5**.

identified by 2D NMR and comparison with the published NMR data[8] (see Supporting Information, some assignments corrected). The only previous reports on **2** are of its occurrence in the related brown algae *P. caudata*[8,10] and *S. pedunculatus*[11] (see above).

Compound **5** had the formula $C_{16}H_{20}O_2$ by HRESIMS. 2D NMR data (Supporting Information) showed the presence of a 1,1-dimethylprop-2-enyl chain attached to an aromatic ring, with NMR signals similar to those of compound **4** (Table 1). HMBC correlations defined the signal of the aromatic carbon bearing this chain (C-5, Figure 2). This C-5 signal also showed an HMBC correlation from an OH singlet (C-4-OH, Figure 2), placing this *ortho* to the 1,1-dimethylprop-2-enyl chain. This OH signal showed another HMBC correlation to an aromatic CH, placing this *ortho* to the C-OH (CH-3, Figure 2). Since this and the other aromatic ring proton did not show any coupling, they were placed *para* to each other (CH-6, Figure 2). HMBC correlations between CH-3 and an olefinic CH placed this *ortho* (CH-1″, Figure 2). This left the second oxygen atom at the *para* position to C4-OH (C1-O, Figure 2). This second oxygen atom was attached to a quaternary carbon (75.8 ppm), which showed HMBC correlations from the olefinic protons and from two equivalent methyl groups (C-3″, Figure 2). These linkages gave the proposed structure **5**, previously unreported, but supported by the fungal metabolite **6** showing very similar NMR shifts for analogous atoms.[17]

This discovery of compounds **4** (yield approximately 1% w/w from dried alga), **2** (0.8%), and **5** (0.2%) from *P. capillaris* extends the examples of bis-prenylated phenols as chemotaxonomic markers (along with **1** and **3**) found only in brown algae of the order Sporochnales. Compounds **1**–**5** could be biosynthesized by simple steps from a common precursor such as 2-(3-methylbut-2-enyl)phenol, which has been synthesized[18] but has not been reported as naturally occurring.

Quinone **4** was the main anti-inflammatory compound in the *P. caudata* extract, inhibiting superoxide production by human neutrophils[13] with a mean IC_{50} of 2.1 μM (standard error of the mean 1.4, $n = 3$). The other two isolated compounds were less active in this assay: phenol **2** IC_{50} 29 μM (SEM 3.1, $n = 3$); phenol **5** IC_{50} 238 μM (SEM 166, $n = 3$). However, quinone **4** was more active in an antiproliferative assay using HL60 human leukemia cells, with a mean IC_{50} of 0.34 μM (SEM 0.03, $n = 4$). This is similar to the HL60 IC_{50} of 0.64 μM reported for the simpler 2,5-dimethyl *p*-benzoquinone.[19] The phenols were weaker in their antiproliferative activity: **5** IC_{50} 5.6 μM (SEM 2.4, $n = 3$); **2** IC_{50} 2.7 μM (SEM 0.5, $n = 2$). This indication of potential toxicity of quinone **4** contrasts with results on a more complex, fully substituted quinone **7**. The tunicate natural product **7** showed selective anti-inflammatory activity *in vitro* (anti-inflammatory IC_{50} 1.5 μM, antiproliferative IC_{50} 73 μM) and *in vivo* activity in a gout model.[2] We are not pursuing quinone **4** as an anti-inflammatory lead, but the antiproliferative activity may be worthy of further investigation, since monoprenylated hydroquinone **8** showed anticancer activity *in vivo*.[20]

Experimental Section

General Experimental Procedures. These were carried out as previously described.[21]

Collection and Screening. *Perithalia capillaris* was collected from Southwest Island, Three Kings Island, on November 25, 2002, by scuba at 7 m depth. Identification was made by Dr. Wendy Nelson (NIWA, Wellington) using morphological and microscopic techniques. A voucher is held by NIWA (collection code MNP7070). The initial extract for screening was prepared as described elsewhere.[1]

Bioactivity-Directed Isolation of 4 and Isolation of 2 and 5. Dried *P. capillaris* (42 g) was ground to a fine powder, then shaken overnight in $MeOH/CH_2Cl_2$ (3:1, 400 mL). The extract was filtered and evaporated *in vacuo* to give a green solid (5.49 g). A portion of the extract (1 g) was separated by Si gel column chromatography, eluting with *n*-hexane, then increasing concentrations of $CHCl_3$, EtOAc, and then MeOH. The most anti-inflammatory fraction ($CHCl_3$, 72 mg) was predominantly compound **4** (>95% pure by ^{1}H NMR). Combined fractions eluted with $CHCl_3$ to 1:1 $CHCl_3$/EtOAc (192 mg) were separated on a second Si gel column eluting with *n*-hexane and then increasing concentrations of $CHCl_3$. A fraction eluted with 1:3 *n*-hexane/$CHCl_3$ was predominantly compound **2** (58 mg, approximately 10% of **5** by ^{1}H NMR). A fraction from this second column eluted with 1:1 *n*-hexane/$CHCl_3$ (41 mg) was separated on a third Si gel column eluting with petroleum ether (bp 40–60 °C) and then increasing concentrations of CH_2Cl_2. A fraction eluted with 3:1 petroleum ether/CH_2Cl_2 was predominantly compound **5** (19 mg, approximately 25% of **5** by ^{1}H NMR).

5-(1,1-Dimethylprop-2-enyl)-2-(3-methylbut-2-enyl)cyclohexa-2,5-diene-1,4-dione (4): yellow oil; UV (MeOH) λ_{max} (log ε) 253 (4.10) nm; IR (film) ν_{max} 2969, 2928, 1659, 1598, 1337, 1231, 913, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HREIMS *m/z* 244.1463 [M]$^+$ (13, calcd for $C_{16}H_{20}O_2$ 244.1463), 229.1228 (50), 201.0925 (89), 69.0043 (100).

4-(1,1-Dimethyl-2-propenyl)-2-(3-methyl-2-butenyl)phenol (2) [73215-04-0]: yellow oil; UV (MeOH) λ_{max} (log ε) 277 (3.38) 225 sh (4.00) nm; IR (film) ν_{max} 3447, 2967, 1630, 1504, 1263, 1114, 911

cm^{-1}; ^{1}H and ^{13}C NMR data, Supporting Information; all matching Blackman et al.[8]

2,2-Dimethyl-7-(1,1-dimethylprop-2-enyl)-2*H*-chromen-6-ol (5): yellow oil; UV (MeOH) λ_{max} (log ε) 332 (3.31), 266 (3.31), 231 (4.03) nm; IR (film) ν_{max} 3490, 2971, 2929, 1635, 1493, 1424, 1360, 1321, 1263, 1251, 1213, 1170, 1111, 908 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 267.1364 [MNa]$^+$ (calcd for $C_{16}H_{22}O_2Na$ 267.1361).

Biological Assays. The superoxide assay was carried out as previously described using human neutrophils with the respiratory burst triggered by phorbol 12-myristate 13-acetate.[13] For the antiproliferative assay, HL60 cells were used as previously described.[22]

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Supporting Information Available: Tables of 2D NMR data for **2**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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