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

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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

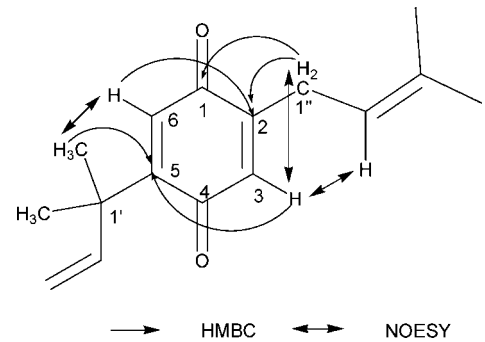

Figure 1. Key 2D NMR correlations for compound **4**.

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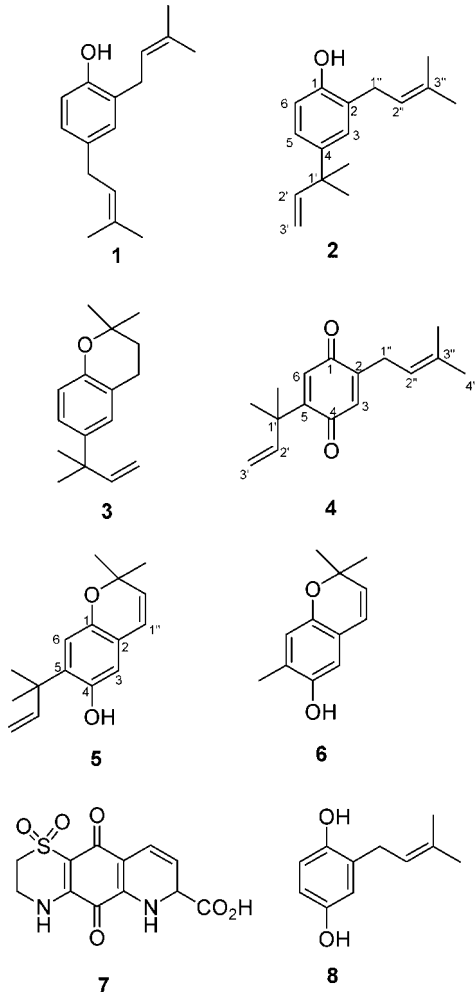

identified by 2D NMR and comparison with the published NMR data[8] (see Supporting Information, some assignments corrected). The only previous reports on **2** are of its occurrence in the related brown algae *P. caudata*[8,10]and *S. pedunculatus*[11] (see above).

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.

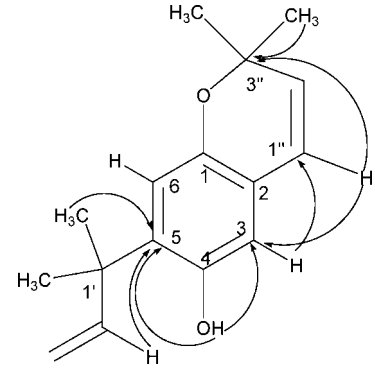

Figure 2. Key HMBC NMR correlations for compound **5**.

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}; ^1H 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}; ^1H 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: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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