Anti-inflammatory Sesquiterpene-quinones from the New Zealand Sponge *Dysidea* cf. *cristagalli*

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The inhibition of superoxide production by human neutrophils has been used to screen New Zealand's unique biota for anti-inflammatory natural products. Bioactivity-directed isolation on an extract of the sponge *Dysidea* cf. *cristagalli* led to a new sesquiterpene-quinone (**4**) with anti-inflammatory activity, plus acetylated hydroquinone (**3**). These compounds inhibited superoxide production in vitro with IC_{50}'s of 3 μM (**3**) and 11 μM (**4**).

New anti-inflammatory pharmaceuticals are needed to treat diseases such as arthritis[1,2] and chronic obstructive pulmonary disease.[3] Superoxide production is recognized as an important stage of the inflammatory response,[4,5] preceding the usual pharmacological targets of phospholipase[6] and cyclooxygenase[7] enzymes. We are searching for new classes of anti-inflammatory natural products by screening for inhibition of superoxide production by human neutrophils.[8]

Screening of New Zealand's terrestrial plants and marine organisms led us to extracts of the sponge *Dysidea* cf. *cristagalli* Bergquist, 1961 (order Dictyoceratida, family Dysideidae), which gave reproducible inhibition of superoxide production. There are no previous records of compounds isolated from this species, despite many reports of a wide range of natural products from sponges in the genus *Dysidea*.[9] A recent review on anti-inflammatory metabolites from sponges[10] highlights a series of sesquiterpene-quinones from *Dysidea*. The first found were the rearranged drimane-quinones avarol (**1**) and avarone (**2**),[11] which exhibit in vitro and in vivo anti-inflammatory activities.[12] We now describe the bioactivity-directed isolation, structure identification, and anti-inflammatory activities of acetylated hydroquinone (**3**) and the new sesquiterpene-quinone (**4**) from *Dysidea* cf. *cristagalli*.

The sponge extract was fractionated using reversed-phase (C18) and silica gel chromatography, directed by the assay for inhibition of superoxide production. This led to two main anti-inflammatory compounds: **3** (isolated yield 0.1% w/w from the wet sponge) and **4** (0.03%).

Full spectroscopic analyses led us to propose the structure shown for compound **3**. During this work, Perez-Garcia et al. reported compound **3** from two unidentified *Dysidea* sponges collected from the Gulf of California, Mexico.[13] Our data and assignments matched theirs on all counts including optical rotation, showing the same absolute stereochemistry. The aromatic moiety of compound **3** has been reported in only one other compound, from *D. avara*, assigned structure **5**.[14] The NMR data for **3** (Supporting Information) closely match the corresponding signals reported by De Giulio et al. for compound **5**.[14] However, we note that the ^{1}H and ^{13}C NMR data reported for structure **5** are very similar (all but one ^{13}C signal within ±0.1 ppm, allowing for different assignments) to the data reported for a compound from *D. cinerea*, which was assigned the regioisomeric 6′-*O*-acetyl structure **6** by Hirsch et al.[15] The optical rotations of **5** and **6** are also similar, at +16° and +18.9°, respectively. Therefore these may be the same compound, whose structure should be re-examined.

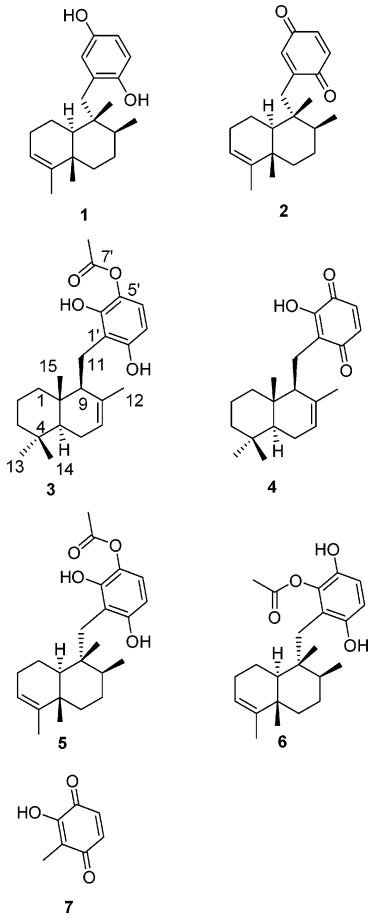

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Table 1. NMR Data for Compound **4**[a]

	^{13}C[b]	^{1}H[c]
1ax	39.1	1.24, td, NR
1eq		1.81, m, NR
2ax	19.0	1.51, qt, 13.5, 2.5
2eq		1.43, m, 3.5
3ax	42.1	1.17, td, 13, 3
3eq		1.38, dm, 15, 2.5
4	33.0	
5	50.1	1.21, dd, 13, 5
6eq	23.7	1.88, br d, 17
6ax		1.83, m, NR
7	122.9	5.39, dt, 2, 1.5
8	134.7	
9	51.8	2.46−2.54, m
10	37.3	
11a	21.6	2.46−2.54, m
11b		2.46−2.54, m
12	22.4	1.55, m
13eq	33.3	0.84, s
14ax	22.0	0.88, s
15	13.5	0.85, s
1′	123.8	
2′	187.7	
3′	131.6	6.72, d, 10
4′	139.5	6.69, d, 10
5′	182.8	
6′	150.8	
6′-OH		7.00, s

[a] In $CDCl_3$. [b] At 125 MHz: shift in ppm. [c] At 500 MHz: shift in ppm, couplings in Hz.

Compound **4** from *D.* cf. *cristagalli* had the molecular formula $C_{21}H_{28}O_3$ by HREIMS. The IR spectrum showed the presence of both OH (3330 cm^{-1}) and conjugated ketone (1665 and 1626 cm^{-1}) groups. The NMR spectra of compound **4** (Table 1) showed signals appropriate for the same drimane sesquiterpene portion as compound **3**, plus signals consistent with a 1′-alkyl-6′-hydroxy quinone moiety. Surprisingly, only two compounds containing this simple moiety have been reported previously. Compound **7**, from a millipede,[16] had ^{13}C NMR signals well matched by the corresponding signals of compound **4**. 2D NMR data (Supporting Information) were in accord with the proposed structure **4**, which has not previously been reported. The comparable ^{13}C signals of the drimane moieties indicated that the relative stereochemistry of **4** is the same as **3**, and the absolute stereochemistry is assumed to be the same as that established by Perez-Garcia et al. for **3**.[13]

Compounds **3** inhibited superoxide production by human neutrophils with a mean IC_{50} of 3.0 μM (standard deviation 0.2), and **4** had a mean IC_{50} of 11 μM (SD 2). These in vitro potencies are similar to those observed for avarol (**1**) and avarone (**2**),[12,17] but the mechanism of the superoxide inhibition by sesquiterpene-quinones is not known.[10] The lipophilic sesquiterpene moiety will favor accumulation in cell membranes, and the redox-active quinone moiety may interfere with superoxide production at the cell surface. On the other hand, it has been suggested that sesquiterpene-quinones can induce formation of superoxide, leading to cytotoxic effects.[18] Although avarol (**1**) and avarone (**2**) display in vivo anti-inflammatory activities by oral administration,[12] the sesquiterpene-quinone class, including **3** and **4**, lacks the novelty necessary for development of commercial anti-inflammatory agents. In addition, the higher antiproliferative activities of **3** (IC_{50} 0.37 μM, SD 0.02) and **4** (IC_{50} 0.34 μM, SD 0.03), compared with their anti-inflammatory activities (see above), indicate potential for in vivo side effects.

Compounds **3** and **4** are the first reported metabolites from the New Zealand sponge *Dysidea* cf. *cristagalli*, although avarol (**1**) and related compounds have been reported from an unspecified New Zealand *Dysidea* collection.[19] The different quinone moieties of **3** and **4** represent another variation in the biosynthetic combinatorial chemistry[20] that sponges of the genus *Dysidea* have evolved to produce.

Experimental Section

General Experimental Procedures. These were recently described.[21]

Collection and Screening. The sponge was collected by scuba in February 2002 from a reef flat in Spirits Bay, Northland, New Zealand, at 33 m depth. It is most closely comparable to *Dysidea cristagalli* Bergquist, 1961 (order Dictyoceratida, family Dysideidae); a full description is given in the Supporting Information. A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 2004.10.5.1). The initial extract for screening was prepared by steeping finely chopped freeze-dried sponge (5 g) in MeOH (20 mL) overnight at 4 °C. After 24 h, CH_2Cl_2 (20 mL) was added and the extract shaken at room temperature for 3 h followed by filtering (Whatman #1 paper). The filtrate was dried, resuspended in $MeOH/CH_2Cl_2$ (3:1) in a volume equal to the filtrate, and left overnight. The supernatant was dried and a subsample (10 mg) submitted for assay.

Bioactivity-Directed Isolation of 3 and Isolation of 4. The frozen sponge (73 g wet) was homogenized and then shaken overnight in $MeOH/CH_2Cl_2$ (3:1, 500 mL). The extract was filtered and evaporated in vacuo to give a green gum (3 g). A portion (1 g) was separated by C_{18} column chromatography, eluting with H_2O, then increasing concentrations of MeCN, and finally $CHCl_3$. The most anti-inflammatory fraction (80% MeCN, 58 mg) was further separated (51 mg aliquot) by column chromatography over silica gel eluting with $CHCl_3$, then increasing concentrations of MeOH, to give the most anti-inflammatory fraction (2% MeOH, 34 mg) containing compound **3**. Similar separations on the rest of the extract (2 g) gave more of **3** plus compound **4** (eluted from silica gel with $CHCl_3$, 16 mg).

20-*O*-Acetyl-21-hydroxy-*ent*-isozonarol (3): orange gum; $[\alpha]^{19}_D$ −24° (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 284 (3.23) nm; IR (film) ν_{max} 3427, 2922, 2360, 1736, 1604, 1468, 1367, 1217, 1043, 1014, 802, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, see Supporting Information; negative ion ESIMS *m/z* 371 $[M - H]^-$ (100), 329 $[M - OCCH_3]^-$ (100); positive ion EIMS *m/z* 372.2295 $[M]^+$ (30, calcd for $C_{23}H_{32}O_4$ 372.2301), 330 (80), 314 (30), 206 (30), 191 (100), 139 (45), 119 (40), 109 (60).

21-Hydroxy-*ent*-isozonarone (4): yellow semicrystalline solid; $[\alpha]^{19}_D$ −86° (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 254 (3.79), 409 (2.90) nm; IR (film) ν_{max} 3330, 2922, 1665, 1626, 1595, 1456, 1373, 1334, 1196, 1128, 1038, 1000, 836, 668 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; negative ion ESIMS *m/z* 327 $[M - H]^-$ (100); positive ion EIMS *m/z* 328.2034 $[M]^+$ (10, calcd for $C_{21}H_{28}O_3$ 328.2038), 313 (25), 189 (60), 140 (35), 119 (100), 109 (25).

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.[8] For the antiproliferative assay, HL60 cells were used as previously described.[22]

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Supporting Information Available: A full taxonomic description of the sponge, and tables of 2D NMR data for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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