5-epi-Isospongiaquinone, a New Sesquiterpene/Quinone Antibiotic from an Australian Marine Sponge, Spongia hispida

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5-*EPI*-ISOSPONGIAQUINONE, A NEW SESQUITERPENE/QUINONE ANTIBIOTIC FROM AN AUSTRALIAN MARINE SPONGE, *SPONGIA HISPIDA*

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ABSTRACT.—An Australian marine sponge, *Spongia hispida*, has been found to contain a new sesquiterpene/quinone identified by detailed spectroscopic analysis and chemical derivatization as the antibiotic 5-*epi*-isospongiaquinone [**3**]. The complete stereostructure for **3** was determined by detailed spectroscopic analysis and chemical correlation with the known marine natural product isospongiaquinone [**2**]. Co-occurring with **3** was an ethylated analogue, 5-*epi*-homoisospongiaquinone [**4**], which was speculated to be an artifact of the isolation process. A revised structure **15** for the known marine metabolite, smenorthoquinone [**13**], is also presented.

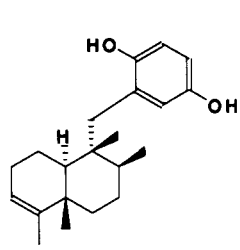
Numerous metabolites of mixed biosynthesis, incorporating a sesquiterpene unit coupled to a quinone or quinol, have been reported from marine sponges (1, R.J. Capon, Marine Sesquiterpene/Quinones, a chapter in "Studies in Natural Products Chemistry: Structure and Chemistry," edited by Atta-ur-Rahman, Elsevier, manuscript in preparation). Among the earliest example were avarol [**1**] (2,3) and isospongiaquinone [**2**] (4,5). Other structural variants include compounds incorporating acyclic, monocyclic, and bicyclic sesquiterpene units, coupled to aromatic moieties of varying oxidation level and substitution. Many of the more than 100 known examples of this structure class have been reported to exhibit antibiotic and/or anticancer activity, while a few have been described as displaying anti-HIV-1 activity (6, 7 and R.J. Capon, manuscript in preparation).

In this report we describe the isolation and structure elucidation of a new sesquiterpene/quinone, 5-*epi*-isospongiaquinone [**3**], along with a possible artifact of the isolation process, 5-*epi*-homoisospongiaquinone [**4**]. We also take the opportunity to revise the structure assigned to smenorthoquinone from *o*-quinone **13** to *p*-quinone **15**.

RESULTS AND DISCUSSION

The crude EtOH extract of a specimen of *Spongia hispida* Lamarck (Dictyoceratida: Spongiidae) collected during trawling operations off Portland along the southwestern coast of Victoria, was found to inhibit significantly the growth of several test bacteria (*Staphylococcus aureus*, a *Micrococcus* sp., and a *Serratia* sp.). The concentrated extract was partitioned as outlined in the Experimental section to yield two pure CH_2Cl_2-soluble antibiotics, **3** and **4**.

The molecular formula determined for **3** by accurate mass measurement ($C_{22}H_{30}O_4$) confirmed it to be isomeric with isospongiaquinone [**2**]. Furthermore, the close similarity between the ^{1}H-nmr data for **3** and that reported for isospongiaquinone [**2**] (5) suggested that **3** was a stereoisomer of **2**. A comparison of the ^{13}C-nmr data for **3** and **2** (Table 1) clearly established that the relative stereochemistry about the ring junction in **3** was cis, rather than trans. In particular, the deshielded resonance attributed to C-12 in **3** (32.3 ppm) relative to **2** (19.9 ppm) was interpreted as being diagnostic of a cis rather than a trans ring junction (8). Acid-catalyzed rearrangement of **3** followed by methylation with CH_2N_2 yielded two products, **5** and **6**, identical in all respects to the acid-catalyzed rearrangement products from isospongiaquinone [**2**]. Thus **3** was identified as 5-*epi*-isospongiaquinone. Stereoisomeric pairs such as 5-*epi*-isospongiaquinone [**3**] and isospongiaquinone [**2**] are not new to marine natural products, with other ex-



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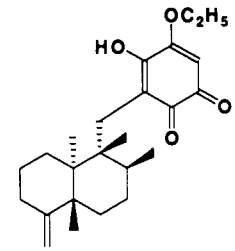

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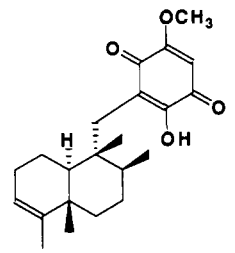

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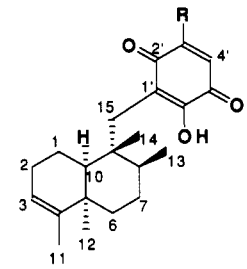
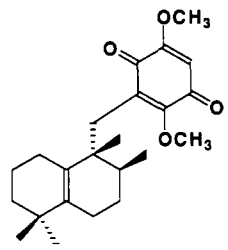


3 R=OMe
4 R=OEt



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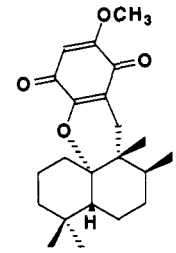
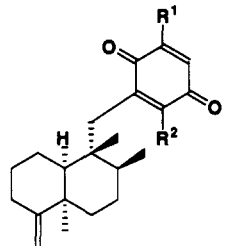


6

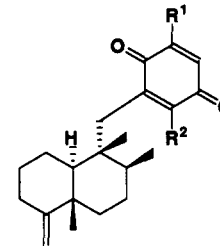
amples including arenarone [**7**] (9) and neoavarone [**8**] (10), 5-*epi*-ilimaquinone [**9**] (11) and ilimaquinone [**10**] (12,13), and arenarol [**11**] (9) and neoavarol [**12**] (10).

Co-occurring with 5-*epi*-isospongiaquinone [**3**] was a homologue **4** ($C_{23}H_{32}O_4$). This compound was spectroscopically very similar to 5-*epi*-isospongiaquinone [**3**], the only significant difference being the replacement of nmr resonances for a methoxy group [δ 3.88 (s); 56.8, (q)] with those for an ethoxy group [δ 1.51 (5), 4.05 (q); 13.8 (q), 65.9 (t)]. These observations were consistent with **4** being assigned the structure 5-*epi*-homoisospongiaquinone as shown. Although one other ethoxylated sesquiterpene/quinone has been reported as a natural product, smenorthoquinone [**13**] (14), we are inclined to believe that **4** is an artifact of the isolation procedure. Under acid-catalyzed conditions in the presence of a surplus of EtOH it is possible for "trans esterification" to transform **3** into **4**. These results prompted us to examine more closely the structure assignment of smenorthoquinone [**13**].

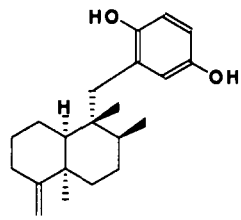
Smenorthoquinone was reported from a *Smenospongia* sp. collected near Djibouti in the Gulf of Aden, and an *o*-quinone structure, **13**, was proposed primarily on the basis of a reaction with *o*-phenylenediamine to produce the adduct **14** (14). A re-examination of both the spectroscopic data for smenorthoquinone and its reactivity with *o*-



7 $R^1=R^2=H$
9 $R^1=Me$, $R^2=OH$



8 $R^1=R^2=H$
10 $R^1=Me$, $R^2=OH$
15 $R^1=OEt$, $R^2=\pm OH$



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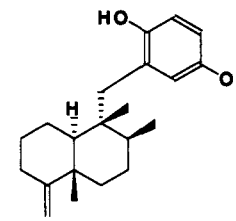
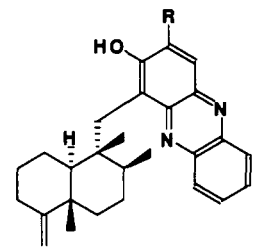


12



14 R=OEt
16 R=OMe

phenylenediamine suggested the need for a structure revision. Spectroscopically, smenorthoquinone was very similar to ilimaquinone [**10**] (12,13), with the exception that rather than incorporating a methoxy substituent on the aromatic ring [δ 3.86 (s), 56.79 ppm (q)], smenorthoquinone possessed an ethoxy substituent [δ 1.49 (t), 4.04 (q); 65.86 (t), 13.72 ppm (q)]. Rather than propose that smenorthoquinone was the homologue **15** of ilimaquinone [**10**], as the spectroscopic data would suggest, the original workers proposed the alternative *o*-quinone structure **13**. This assignment was based primarily on the outcome of a reaction between smenorthoquinone and *o*-phenylenediamine, which yielded an adduct identified as **14**. Although little effort was made to characterize and hence substantiate the structure assigned to this adduct, the formation of an adduct was deemed to be sufficient evidence for the existence of an *o*-quinone moiety. Hence smenorthoquinone was initially assigned structure **13**.

That this assignment was possibly incorrect was apparent from the original data. It would be remarkable if the ^1H- and ^{13}C-nmr data for an *o*-quinone such as **13** were so similar to that for a *p*-quinone such as **10**. Even more anomalous was the fact that the uv maxima reported for the supposed *o*-quinone **13** (209, 283 nm) were virtually identical to those for the known *p*-quinone **10** (209, 286 nm). It is well known that *o*-quinones have uv maxima at a significantly higher wavelength than corresponding *p*-quinones

TABLE 1. ^{13}C-nmr ($CDCl_3$, 100 MHz) Data for Isospongiaquinone [**2**], 5-*epi*-Isospongiaquinone [**3**], and 5-*epi*-Homoisospongiaquinone [**4**].

Carbon	Compound		
	2	**3**[a]	**4**[a]
C-1	17.7	18.2	18.2
C-2	27.1	24.4	24.4
C-3	121.0	123.9	123.9
C-4	143.9	138.9	138.9
C-5	43.1	44.5	44.4
C-6	36.1	37.3	37.3
C-7	28.1	29.1	29.1
C-8	38.1	37.2	37.3
C-9	38.6	38.8	38.9
C-10	48.2	46.0	46.1
C-11	20.2	19.7	19.7
C-12	19.9	32.2	32.3
C-13	18.1	19.1	19.1
C-14	17.3	16.3	16.3
C-15	32.5	32.7	32.9
C-1′	117.8	117.7	117.7
C-2′	182.4[b]	182.5[b]	182.2[b]
C-3′	161.8	161.6	161.5
C-4′	102.0	102.0	102.3
C-5′	182.0[b]	182.2[b]	182.6[b]
C-6′	153.4	153.4	153.2
OCH_3	56.8	56.9	—
OCH_2CH_3	—	—	65.9
OCH_2CH_3	—	—	13.8

[a]Assignments were supported by 2D HETCOSY and DEPT nmr experiments, and by comparison of chemical shifts with those assigned to isospongiaquinone [**2**]. Multiplicities of all resonances are consistent with the assignments indicated in the table.

[b]Assignments with identical superscripts within a column may be interchanged.

(15). In fact, the *o*-quinone **13** and the *p*-quinone **15** are resonance tautomers and, given the large number of examples of this structure class known to prefer *p*-quinone structures, it would appear that the *p*-quinone tautomer is far more stable than the corresponding *o*-quinone tautomer. Furthermore, this tautomerization adequately explains why the "*p*-quinone" smenorthoquinone would yield the adduct **14**. Indeed, just such an observation has been made in the case of ilimaquinone [**10**], which on treatment with *o*-phenylenediamine yielded the corresponding adduct **16** (6). On the basis of the arguments outlined above the structure for smenorthoquinone is revised from the *o*-quinone tautomer **13** to the *p*-tautomer **15**. As smenorthoquinone was found to co-occur with ilimaquinone, the possibility that smenorthoquinone was an artifact of the isolation procedure cannot be entirely excluded.

Both 5-*epi*-isospongiaquinone [**3**] and 5-*epi*-homoisospongiaquinone [**4**] displayed antibiotic activity against *Staphylococcus aureus* (MIC 20 μg/disc and 50 μg/disc, respectively) and a *Micrococcus* sp. (MIC 20 μg/disc and 50 μg/disc, respectively).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Details of experimental procedures are as previously published (16).

COLLECTION, EXTRACTION, AND ISOLATION.—A specimen of *S. hispida* was obtained during

trawling operations off Portland, along the south western coast of Victoria, Australia, and a sample (registry number QMG300711) is lodged with the collection of the Queensland Museum. The frozen sponge was transported to the laboratory where it was diced and steeped in EtOH until required. The crude EtOH extract was decanted, concentrated under reduced pressure, and partitioned into CH_2Cl_2-solubles and CH_2Cl_2-insolubles. Both fractions displayed antibacterial activity. The CH_2Cl_2-soluble fraction was further partitioned by rapid silica filtration (10% stepwise elution from petroleum ether to CH_2Cl_2 to EtOAc) and normal phase hplc (2.0 ml/min, either 10% EtOAc/hexane or 40% EtOAc/hexane on a Phenomenex Spherex 5 μm 10 × 250 mm column) to yield **3** (90 mg, 0.56% extracted dry wt) and **4** (5 mg, 0.03% extracted dry wt).

5-epi-*Isospongiaquinone* [**3**].—A stable yellow/orange oil: $[\alpha]^{20}D$ −41.2° (*c* = 1.08, $CHCl_3$); ir ($CHCl_3$) ν max 3338, 2938, 2851, 1642, 1605, 1230, 1204 cm^{-1}; uv (EtOH) λ max 205 (ε = 12800), 285 (ε = 2000), (EtOH + NaOH) 287 (ε = 4500) nm; ^1H nmr (400 MHz, $CDCl_3$) δ 0.91 and 0.94 (2s, H_3-12 and H_3-14), 0.92 (d, *J* = 7.2 Hz, H_3-13). 0.95–1.12 (m, methylene envelope), 1.64 (s, H_3-11), 1.85–2.20 (m, methylene envelope), 2.50 (1/2 ABq, *J* = 13.7 Hz, H_a-15), 2.65 (1/2 ABq, *J* = 13.7 Hz, H_b-15), 3.88 (s, OMe), 5.32 (bs, H-3), 5.87 (s, H-4′), 7.45 (s, OH); ^{13}C nmr (100 MHz, $CDCl_3$) see Table 1; eims (70 eV) *m/z* $[M]^+$ 358 (2), 191 (48), 189 (6), 168 (50), 121 (28), 107 (37), 95 (100); hrms *m/z* 358.2144 ($C_{22}H_{30}O_4$ requires 358.2144).

5-epi-*Homoisospongiaquinone* [**4**].—A stable pale yellow oil; $[\alpha]^{20}D$ −28.8° (*c* = 0.17, $CHCl_3$); ir ($CHCl_3$) ν max 3337, 2929, 1724, 1642, 1603, 1227 cm^{-1}; uv (EtOH) λ max 205 (ε = 41000), 292 (ε = 11300) nm; ^1H nmr (400 MHz, $CDCl_3$) δ 0.92 and 0.95 (2s, H_3-12 and H_3-14), 0.93 (d, *J* = 7.2 Hz, H_3-13), 1.00–1.05 (m, methylene envelope), 1.51 (t, *J* = 6.9 Hz, CH_3CH_2O), 1.64 (s, H_3-11), 1.85–2.20 (m, methylene envelope), 2.50 (1/2 ABq, *J* = 13.6 Hz, H_a-15), 2.63 (1/2 ABq, *J* = 13.6 Hz, H_b-15), 4.05 (q, *J* = 6.9 Hz, CH_3CH_2O), 5.32 (bs, H-3), 5.84 (s, 4′-H), 7.45 (s, OH); ^{13}C nmr (100 MHz, $CDCl_3$) see Table 1; eims (70 eV) *m/z* $[M]^+$ 372 (1), 205 (2), 191 (45), 189 (11), 182 (50), 149 (19), 135 (13), 121 (28), 119 (17), 109 (27), 107 (36), 105 (12), 95 (100); hrms *m/z* 372.2301 ($C_{23}H_{32}O_4$ requires 372.2300).

ACID-CATALYZED REARRANGEMENT OF *5-epi*-ISOSPONGIAQUINONE [**3**].—A sample of *5-epi*-isospongiaquinone [**3**] (23 mg) in MeOH-HOAc-HCl (1:1:1) (2 ml) was gently refluxed for 2 h, after which it was concentrated under reduced pressure and subjected to hplc purification (2.0 ml/min 20% EtOAc/hexane on a Phenomenex Spherex 5 μm 10 × 250 mm column) to yield **5** (6 mg, 26%) and [**6**] (4 mg, 17%). Compounds **5** and **6** were identical in all respects to those previously described from the acid-catalyzed rearrangement of isospongiaquinone [**2**] (5),.

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