Isometachromin, a new cytotoxic sesquiterpenoid from a deep water sponge of the family Spongiidae [1]

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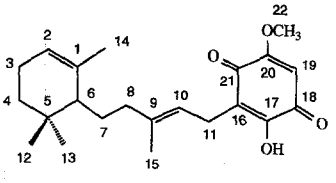
Abstract. Isometachromin (**1**), a new sesquiterpene-quinone that is related structurally to metachromin C (**2**), and the known compounds ilimaquinone (**3**) and and 5-*epi*-ilimaquinone (**4**), were isolated from a deep water sponge in the family Spongiidae; the structure of isometachromin was elucidated by spectral methods. Isometachromin exhibits in vitro cytotoxicity against the human lung cancer cell line A 549 (IC_{50} = 2.6 μg/ml), but not against P388 murine leukemia ($IC_{50} \geq 10$ μg/ml) and also exhibits antimicrobial activity.

Key words. Deep water marine sponge; Spongiidae; cytotoxicity; antimicrobial activity.

Our research on the chemical constituents of shallow and deep water marine organisms has focused in part on the discovery of cytotoxic compounds with therapeutic potential [2–4]. In this note, we report the isolation and identification of isometachromin (**1**), a new sesquiterpene-quinone from a deep water sponge in the family Spongiidae [5], which exhibits in vitro cytotoxicity against the human lung cancer cell line A 549 (IC_{50} = 2.6 μg/ml), but not against P388 murine leukemia ($IC_{50} \geq 10$ μg/ml), and exhibits modest antimicrobial activity, i.e., against *Candida albicans* and *Cryptococcus neoformans* [6]. From this deep water sponge, we also report the isolation of two previously identified terpene-quinones. Isometachromin (**1**) was isolated from a sponge collected near Chub Cay, Bahamas, in December, 1984, at a depth of approximately 800 m using a manned submersible, and then freshly frozen. Extraction of a thawed portion of the sponge (100 g) with MeOH/toluene (3/1) and MeOH yielded a crude extract (combined weight of extracts, 5 g), which was partitioned between water and 1,2-dichloroethane. A portion of the residue (0.37 g) from the 1,2-dichloroethane phase (0.74 g) was chromatographed by multilayer planetary coil countercurrent chromatography [7] (CCC) using a solvent system of heptane/CH_2Cl_2/acetonitrile (10/3/7 – upper phase used as mobile phase) to yield fractions that contained the same mixture of structurally related metabolites as judged by ^{1}H NMR (combined weight, 32 mg). Vacuum liquid chromatography (VLC) of the combined fractions using silica gel as the adsorbent (step gradient of 25–40% $CHCl_3$/heptane) afforded **1** (approximately 0.16% of crude extract, 8×10^{-3}% of frozen organism) as an oil ($[\alpha]_D$ – 9.6° (c 0.08, $CHCl_3$)).

The molecular formula of **1** was deduced as $C_{22}H_{30}O_4$ from high resolution EIMS (*m/z* 358.2151, Δ 0.7 nm), which requires eight degrees of unsaturation. The ^{1}H NMR spectrum of **1** [8] contained methyl signals at δ 0.82 (s, 3H), 0.87 (s, 3H), 1.62 (br s, 3H), and 1.72 (br s, 3H), and olefin protons at 5.11 (br t, 1H, J = 7.3 Hz), and 5.24 (br s, 1 H), which suggested that **1** was terpenoid and consisted of cyclic (with gem-dimethyl and endocyclic olefin groups) and acyclic elements (isoprene group). The ^{13}C NMR resonances observed at δ 151.2 (s), 161.1 (s), 181.9 (s), 183.3 (s), 118.2 (s) and 102.2 (d), and the ^{1}H NMR resonances observed at δ 3.83 (s, 3H), 5.81 (s, 1H) and 7.20 (s, 1H, D_2O exchangeable) suggested the presence of a monomethyl ether of an alkyl-substituted-dihydroxybenzoquinone group [9a]; further evidence for the presence of a hydroxybenzoquinone group in **1** was obtained from UV [10] and IR data [9b] (λ_{max}(MeOH, nm) 210 (ε 11,500), 288 (ε 12,500) and 427 (ε 600), and 3340, 1660, 1640, and 1610 cm^{-1}, respectively). The structural similarity of **1** with metachromin C (**2**)[11] was recognized. Comparison of NMR data between **1** and **2** revealed that half of the resonances observed for **1** [8], i.e., resonances for C9–C11, C15–C22, and for H10, H11, H15, H19, H22, and (H17) O*H*, are virtually identical to those reported for **2** [11]. The partial structure defined by these resonances accounts for six of the eight degrees of unsaturation in **1**; because **1** contains only one additional (trisubstituted) double bond (^{13}C NMR resonances observed at δ 119.8 (d) and 136.8 (s); ^{1}H NMR resonances observed at 5.24 (br s, 1H) and 1.62 (br s, 3H)), it must contain an addi-



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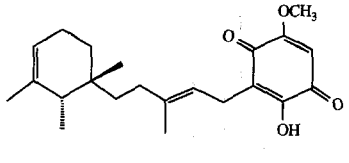

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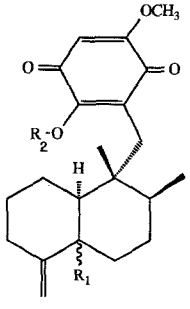

	R_1	R_2
3	β CH_3	H
4	α CH_3	H
5	β CH_3	Ac
6	α CH_3	Ac

tional ring. The completion of the structure elucidation of **1**, as an unrearranged monocyclofarnesol unit, was achieved by interpretation of NMR data derived from HMQC[12], HMBC[13], selective INEPT[14], COSY[15] (including COSY long-range), homonuclear decoupling, and nOe difference[16] experiments: H8 + H3 (overlapping proton resonances)/C1, C2, C4, C5 + C6, C7, C9, C10, C15; H8/H10, H15; nOe between H8 and H10 (1%); H12 or H13/C4, C5, C6; H12 or H13/H4 (one of the two H4 protons) or H6 (both protons at δ 1.38); H14/C1, C2, C6; H14/H2; nOe between H14 and H2 (2.4%); H2/C3, C4, C6, C14; H2/H3, H14; H3/H2, H4. The nOe between H8 and H10 confirmed the *E*-geometry around the C9–C10 double bond. A noteworthy ^{1}H NMR spectral feature of **1** is the unusual high-field (shielded) chemical shift of the allylic proton at C-6, i.e., δ 1.38; an analogous allylic methine proton in metachromin C (**2**) is observed at δ 1.65. The observation that isometachromin is optically active is consistent with the structure proposed (**1**); however, the absolute configuration of **1** has not been determined. Biogenetically, isometachromin (**1**) is probably derived from an arylated farnesol precursor through protonation of its distal double bond, followed by an attack of the central double bond and subsequent proton elimination. The isomeric metabolite metachromin C (**2**) appears to be related to **1** through a series of carbonium ion-induced alkyl shifts and proton elimination.

Several previously identified terpene-quinones were isolated from VLC fractions that yielded **1**. The ^{1}H NMR spectrum of material from which **1** had been purified also showed several signals at δ 5.8 and 3.8 in approximately 1:3 ratios; however, the mixture lacked the resonances observed for **1** at δ 3.18 (d, J = 7.3 Hz), and, instead showed resonances (broad singlets) at δ 4.70, 4.67, 4.44, and 4.43, in ratios of 2:2:1:1, which suggested the presence of ilimaquinone[17,18] (**3**) and the structurally related compound 5-*epi*-ilimaquinone[19] (**4**). Because attempts to separate these compounds were unsuccessful, a slightly modified protocol of Carte et al.[19] was followed whereby the mixture was acetylated and the acetates were subjected to HPLC on silica gel (8% EtOAc/heptane, 5 µ silica gel). Ilimaquinone acetate (**5**) and 5-*epi*-ilimaquinone acetate (**6**) were purified from this mixture, and found to be identical in all respects to the previously reported compounds[17,19,20].

The mixture of **3** and **4**, before and after acetylation (to yield **5** and **6**) also expressed selective cytotoxicity with IC_{50} values against the A 549 human lung cancer cell line of 7 and 4 µg/ml, respectively, and IC_{50} values against P 388 of $\geq$ 10 µg/ml.

Because the natural products chemistry of deep water sponges is poorly understood, it is noteworthy that compounds **3** and **4** studied in this report, which most likely are produced by a *Spongia* or *Hippospongia* sp.[5], are identical to those isolated from the shallow water Hawaiian sponge, *Hippospongia metachromia*[17], and the shallow water Indo-Pacific sponges, *Fenestraspongia* sp.[19], and *Hippospongia* sp.[21].

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5 The sponge has been identified as either a *Spongia* or *Hippospongia* sp. per Berquist, P. A., New Zealand J. Zool. *7* (1980) 443. The sample number is 13-XII-84-1-37B, and a taxonomic voucher specimen is deposited in the Harbor Branch Oceanographic Museum.

6 *B. subtilis* minimum inhibitory concentration (MIC) = 12.5 µg/ml; *Candida albicans* MIC (using RPMI medium) = 6.2 µg/ml; *Cryptococcus neoformans* MIC = 25 µg/ml; MIC's against *E. coli*, and *C. albicans* (SDB medium) > 50 µg/ml.

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8 Complete spectral data for **1**: $[\alpha]_D$ – 9.6° (c 0.08, $CHCl_3$); UV (MeOH) 210 nm (ε 11,500), 288 (ε 12,500), 427 (ε 600); IR (KBr) 3340, 1660, 1640, 1610, 1380, 1310, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s, H12 or H13), 0.89 (3H, s, H12 or H13), 1.08 (1H, br dt, J = 13,5 Hz, H4)/1.38 (1H, m, H4), 1.28/1.45 (2H, m, H7), 1.38 (1H, m, H6), 1.62 (3H, br s, H14), 1.72 (3H, br s, H15), 1.91 (2H, m, H3), 1.96 (2H, br t, J = 8 Hz, H8), 3.18 (2H, d, J = 7.3 Hz, H11), 3.83 (3H, s, H22), 5.11 (1H, br t, J = 7.3 Hz, H10), 5.24 (1H, br s, H2), 5.81 (1H, s, H19), 7.21 (1H, s, O*H*); ^{13}C NMR ($CDCl_3$) δ 183.3 (s, C18), 181.9 (s, C21), 161.1 (s, C20), 151.2 (s, C17), 138.1 (s, C9), 136.8 (s, C1), 119.8 (d, C2), 119.2 (d, C10), 118.2 (s, C16), 102.2 (d, C19), 56.7 (q, C22), 49.0 (d, C6), 40.5 (t, C8), 32.6 (s, C5), 31.6 (t, C4), 29.7 (t, C7), 27.5 (q, C12), 27.4 (q, C13), 23.5 (q, C14), 23.1 (t, C3), 21.8 (t, C11), 16.26 (q, C15); HREIMS, obsd. m/z 358.2151, $C_{20}H_{30}O_4$ requires 358.2144; LREIMS *m/z* (rel. abund., %) 358(34), 247(13), 234(54), 219(60), 207(61), 189(48), 168(72), 136(100), 121(76), 95(58), 81(87).

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20 Ilimaquinone acetate (**6**): $[\alpha]_D$ – 10.4° (c 0.16, $CHCl_3$) (lit.[13]: $[\alpha]_D$ – 8.3° (c 1.05, $CHCl_3$). 5-*epi*-Ilimaquinone acetate (**7**): $[\alpha]_D$ + 32.3° (c 0.1, $CHCl_3$) (lit.[13]: $[\alpha]_D$ + 22.6° (c 0.95, $CHCl_3$). All spectral data (NMR, UV, IR, MS) were found to be virtually identical with data reported[17,19]. Based on ^{1}H NMR data of the mixtures of **3** and **4**, and **5** and **6**, the concentrations of **3** and **4** in the sponge are 2.7% and 1.4% by weight of the crude extract, respectively, or 0.14% and 0.07% by weight of the frozen sponge, respectively.

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