New Sesquiterpenoid Hydroquinone and Quinones from the Okinawan Marine Sponge (*Dysidea* sp.)

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A new sesquiterpenoid hydroquinone 2 and three new sesquiterpenoid quinones 4, 5 and 6 were isolated from the Okinawan marine sponge, *Dysidea* sp., together with the known hydroquinone 1 and its corresponding quinone 3. The structures of these compounds were elucidated on the basis of spectroscopic data and chemical reactions.

Keywords marine sponge; *Dysidea* sp.; sesquiterpenoid hydroquinone; sesquiterpenoid quinone; rearranged drimane skeleton; avarol; neoavarol; neoavarone; 4′-methoxyavarone; 4′-methoxyneoavarone

Recently a variety of natural products have been isolated from marine sponges.[1] Many of the sponge-derived natural products have received much interest owing to their unique structural features and biological activities. In the course of our studies on the chemical constituents of Okinawan marine animals,[2] we have isolated a new sesquiterpenoid hydroquinone **2** and three new sesquiterpenoid quinones **4**, **5** and **6** from the sponge of *Dysidea* sp. together with the related known compounds **1** and **3**. This paper describes the isolation and structure elucidation of these compounds on the basis of spectroscopic data and chemical reactions.

Extraction and Isolation The methanol extract (13.5 g) of the sponges (wet weight 380 g) of *Dysidea* sp.,[3] collected at the coral reef of Ishigaki Island (Okinawa, Japan), was extracted with a mixture of methylene chloride and ethyl acetate (1 : 1). The methylene chloride–ethyl acetate-soluble portion (2.7 g) was chromatographed on a silica gel column by elution with hexane, hexane–ethyl acetate (10 : 1 and then 2 : 1) and ethyl acetate to give five fractions. The 4th fraction (eluted with hexane : ethyl acetate = 2 : 1) was further subjected to silica gel chromatography followed by high-pressure liquid chromatography (HPLC) (octadecylsilica gel) to give avarol (**1**)[4] (363 mg), which was previously isolated from the Mediterranean sponge *Dysidea avara*, and **2** (71 mg) named neoavarol. The 2nd fraction (eluted with hexane : ethyl acetate = 10 : 1) was chromatographed on a silica gel column and then on a silver(I) nitrate-impregnated silica gel column to give avarone (**3**)[4] (18 mg), which was also isolated from *D. avara*, and **4** (2 mg) named neoavarone. The 3rd fraction (eluted with hexane : ethyl acetate = 2 : 1) was also subjected to silica gel column chromatography followed by HPLC to give **5** (5 mg) and **6** (7 mg), named 4′-methoxyavarone and 4′-methoxyneoavarone, respectively. Compounds **1** and **2** showed ichthyotoxic activity against killifish, *Oryzias latipes* (minimum lethal concentration 20 μg/ml for each compound).

Structures of Neoavarol (2) and Neoavarone (4) Neoavarol (**2**) (molecular weight 314) showed a positive ferric chloride test and an ultraviolet (UV) absorption maximum at 298 nm (ε 3700), indicating the presence of a phenolic group. The proton nuclear magnetic resonance (^{1}H-NMR) (Table I) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of **2** showed the signals due to three methyl groups [^{1}H-NMR δ: 0.86 (3H, s), 1.00 (3H, d, J = 6.1 Hz), 1.06 (3H, s)], a benzylic methylene group [^{1}H-NMR δ: 2.51 (1H, d, J = 14.3 Hz), 2.63 (1H, d, J =

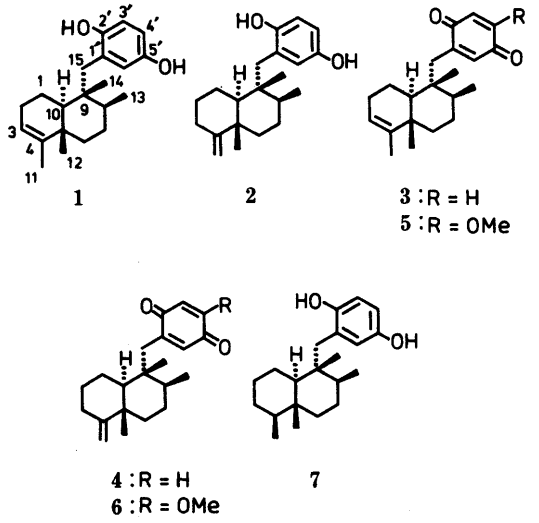

Chart 1

TABLE I. ^{1}H-NMR Data for **1**—**6** (400 MHz, $CDCl_3$, J in Hz)

Proton	1	2	3	4	5	6
3	5.14 (1H, brs)		5.13 (1H, brs)		5.14 (1H, brs)	
11	1.51 (3H, d, J=1.4)	4.39 (1H, brs) 4.43 (1H, brs)	1.53 (3H, brs)	4.44 (1H, brs) 4.46 (1H, brs)	1.53 (3H, s)	4.45 (1H, brs) 4.46 (1H, brs)
12	1.02 (3H, s)	1.06 (3H, s)	1.00 (3H, s)	1.05 (3H, s)	1.00 (3H, s)	1.05 (3H, s)
13	1.00 (3H, d, J=6.4)	1.00 (3H, d, J=6.1)	0.93 (3H, d, J=6.6)	0.93 (3H, d, J=6.5)	0.90 (3H, d, J=6.4)	0.93 (3H, d, J=6.4)
14	0.86 (3H, s)	0.86 (3H, s)	0.85 (3H, s)	0.86 (3H, s)	0.85 (3H, s)	0.86 (3H, s)
15	2.58 (1H, d, J=14.2) 2.67 (1H, d, J=14.2)	2.51 (1H, d, J=14.3) 2.63 (1H, d, J=14.3)	2.44 (1H, d, J=13.4) 2.64 (1H, d, J=13.4)	2.40 (1H, d, J=13.6) 2.57 (1H, d, J=13.6)	2.43 (1H, d, J=13.4) 2.66 (1H, d, J=13.4)	2.40 (1H, d, J=13.1) 2.59 (1H, d, J=13.1)
3′	6.60 (1H, d, J=8.5)	6.59 (1H, d, J=7.2)	6.75 (1H, d, J=10.1)	6.75 (1H, d, J=10)	5.91 (1H, s)	5.90 (1H, s)
4′	6.54 (1H, dd, J=2.9, 8.5)	6.54 (1H, dd, J=2.9, 7.2)	6.70 (1H, dd, J=2.4, 10.1)	6.70 (1H, dd, J=2.4, 10)		
6′	6.59 (1H, d, J=2.9)	6.53 (1H, d, J=2.9)	6.51 (1H, d, J=2.4)	6.45 (1H, d, J=2.4)	6.46 (1H, s)	6.41 (1H, s)
4′-OMe					3.81 (3H, s)	3.61 (3H, s)

14.3 Hz)], an exomethylene group [^{1}H-NMR δ: 4.39 (1H, br s), 4.43 (1H, br s); ^{13}C-NMR δ: 102.9 (t)], and a phenolic group [^{1}H-NMR δ: 6.53 (1H, d, J=2.9 Hz, H-6′), 6.54 (1H, dd, J=2.9, 7.2 Hz, H-4′), 6.59 (1H, d, J=7.2 Hz, H-3′).

These NMR data of **2** are closely related to those of avarol (**1**) as shown in Table I, except for the lack of both the olefinic methyl signal and olefinic proton signal assigned to H-3, and the appearance of the exomethylene signals instead. These findings clearly indicated that neoavarol has the structure represented by **2**. This was confirmed by the following chemical reactions. Hydrogenation of **2** over 10% palladium on carbon gave exclusively the dihydro compound **7**, $[\alpha]_D$ −11.6° (c=0.16, $CHCl_3$), ^{1}H-NMR δ: 0.66 (3H, d, J=6.7 Hz), 0.79 (3H, s), 0.82 (3H, s), 0.98 (3H, d, J=6.3 Hz). Similar hydrogenation of **1** also gave exclusively the same dihydro compound **7**, $[\alpha]_D$ −12.9° (c=0.16, $CHCl_3$). From this finding the structure of neoavarol was elucidated to be **2**, and the absolute structure of neoavarol was also established as **2**, since the absolute structure of **1** has already been determined.[4] In **7**, the β-configuration of the methyl group at C-4 was deduced from the preferential attack of the hydrogen atoms from the less hindered side of **1** and **2**.

The infrared (IR) and UV spectra of neoavarone (**4**) [molecular weight 312, IR 1685 cm^{-1}, UV 247 nm (ε 14900)] showed the presence of a conjugated enone moiety. The ^{1}H-NMR data of **4** are closely related to those of neoavarol (**2**) as shown in Table I, except for the chemical shift and coupling constants of the olefinic protons [δ: 6.45 (1H, d, J=2.4 Hz), 6.70 (1H, dd, J=2.4, 10 Hz), 6.75 (1H, d, J=10 Hz)]. These findings suggested that **4** is the quinone of neoavarol (**2**). This was confirmed by chemical reaction. Oxidation of **2** with silver(I) oxide in ether gave the quinone, whose physical data including optical rotation were identical with those of **4**. Thus, the structure of neoavarone was elucidated to be **4**.

Structures of 4′-Methoxyavarone (5) and 4′-Methoxyneoavarone (6) Compound **5** (molecular weight 342) showed UV absorption at 270 nm (ε 7980) and IR absorption at 1672 and 1649 cm^{-1} attributed to a benzoquinone moiety. The ^{1}H-NMR spectrum of **5** was very similar to that of neoavarone (**3**), except for the lack of one of the three olefinic proton signals in the benzoquinone moiety, and the appearance of the methoxy signal [δ: 3.81 (3H, s)]. The position of the methoxy group was elucidated to be 4′ on the benzoquinone moiety from the fact that no coupling was observed between the olefinic protons of the benzoquinone moiety [δ: 5.91 (1H, s), 6.46 (1H, s)]. Thus the structure of **5** was assigned as 4′-methoxyavarone. Similarly the structure of **6** (molecular weight 342) was assigned as 4′-methoxyneoavarone on the basis of spectroscopic analysis involving the comparison of the spectral data with those of neoavarone (**4**). The absolute structures of **5** and **6** were suggested to be the same as those of **2** and **4**, since **5** and **6** appeared to be biosynthesized from **2** and **4**, respectively, which coexisted with **5** and **6** in the present sponge.

The structures of the present new compounds **2**, **4**, **5** and **6** as well as **1** and **3** are characterized by a rearranged drimane skeleton, which is very rare[5] in natural products from sources other than marine sponges. Sponge-derived sesquiterpenoid hydroquinones and quinones of this type, such as avarol,[6] arenarol,[7] and smenospongine[8] have been reported to show antileukemic activities. Furthermore avarol (**1**) has recently received attention as a possible anti-human immunodeficiency virus (HIV) agent.[9] From these viewpoints, it is of interest to examine further the biological activities of the present compounds **2**, **4**, **5** and **6**.

Experimental

Melting points were measured on a Kofler block and are uncorrected. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1710 spectrophotometer and UV spectra with a Hitachi 124 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Electron-impact mass spectra (EI-MS) were taken with a Hitachi M-80 spectrometer. Column chromatography was carried out on Fuji Davison Silica gel BW-820MH (70—200 mesh). HPLC was conducted with a YMC HPLC-8502 apparatus using YMC-Pack D-ODS-5 (octadecylsilica gel) as a reversed-phase column.

Extraction and Isolation Wet specimens of *Dysidea* sp. (380 g), collected on the coral reef of Ishigaki Island in March, 1988, were extracted with methanol for 2 d. The methanol extract (13.5 g) was suspended in water and extracted with a mixture of methylene chloride and ethyl acetate (1 : 1). The methylene chloride–ethyl acetate-soluble portion (2.7 g), which showed ichthyotoxic activity against killifish (*Oryzias latipes*) at the concentration of 100 μg/ml, was chromatographed on a silica gel column (100 g). Stepwise elution with hexane, hexane–ethyl acetate (10 : 1 and then 2 : 1), and ethyl acetate gave five fractions.

The 4th fraction (1.5 g, eluted with hexane : ethyl acetate = 2 : 1) was further subjected to silica gel column chromatography (hexane : ethyl acetate = 4 : 1 as an eluent) followed by HPLC (methanol : H_2O = 4 : 1 as an eluent) to give avarol (**1**) (363 mg) and neoavarol (**2**) (71 mg). The 2nd fraction (191 mg, eluted with hexane : ethyl acetate = 10 : 1) was also subjected to silica gel column chromatography (hexane : ether = 8 : 1 as an eluent) followed by 10% $AgNO_3$-impregnated silica gel column chromatography (hexane : ethyl acetate = 30 : 1 as an eluent) to give avarone (**3**) (18 mg) and neoavarone (**4**) (2 mg). From the 3rd fraction (103 mg, eluted with hexane : ethyl acetate = 2 : 1), 4′-methoxyavarone (**5**) (5 mg) and 4′-methoxyneoavarone (**6**) (7 mg) were isolated by silica gel column chromatography (hexane : ether = 4 : 1 as an eluent) followed by HPLC (methanol : H_2O = 10 : 1 as an eluent).

Avarol (**1**) and neoavarol (**2**) showed ichthyotoxic activity against killifish, *Oryzias latipes* (minimum lethal concentration 20 μg/ml for each compound).

Avarol (1) Colorless needles. mp 138—140 °C. $[\alpha]_D$ +4.7° (c=0.17, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 298 (3800). EI-MS m/z: 314 (M^+). The data of **1** are in good accordance with those of avarol in the literature.[4]

Neoavarol (2) Colorless needles. mp 151—153 °C. $[\alpha]_D$ −38.6° (c=0.14, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 298 (3700). IR ν_{max}^{KBr} cm^{-1}: 3279, 1631, 1560, 1397, 1189, 898. ^{13}C-NMR ($CDCl_3$) δ: 17.7 (q, 2C), 20.7 (q), 23.3 (t), 27.9 (t), 28.4 (t), 33.1 (t), 36.5 (d), 36.7 (t), 37.7 (t), 40.4 (s), 42.2 (s), 48.3 (d), 102.9 (t), 114.0 (d), 116.3 (d), 119.5 (d), 126.6 (s), 148.8 (s, 2C), 160.1 (s). EI-MS m/z: 314 (M^+). High-resolution MS Calcd for $C_{21}H_{30}O_2$ (M^+): 314.2244. Found: 314.2256.

Avarone (3) Yellow oil. $[\alpha]_D$ +19.1° (c=0.37, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 247 (14700). EI-MS m/z: 312 (M^+). The data of **3** are in good accordance with those of avarone in the literature.[4]

Neoavarone (4) Yellow crystals. mp 78—79 °C. $[\alpha]_D$ −55.2° (c=0.07, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 247 (14900). IR ν_{max}^{neat} cm^{-1}: 1685. EI-MS m/z: 312 (M^+). High-resolution MS Calcd for $C_{21}H_{28}O_2$ (M^+): 312.2088. Found: 312.2090.

4′-Methoxyavarone (5) Yellow crystals. mp 150—152 °C. $[\alpha]_D$ +16.4° (c=0.15, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 270 (7980). IR ν_{max}^{KBr} cm^{-1}: 1672, 1649, 1597. EI-MS m/z: 342 (M^+).

4′-Methoxyneoavarone (6) Yellow crystals. mp 161—163 °C. $[\alpha]_D$ −8.1° (c=0.22, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 270 (7900). EI-MS m/z: 342 (M^+). High-resolution MS Calcd for $C_{22}H_{30}O_3$: 342.2192. Found: 342.2183.

Catalytic Hydrogenation of 2 A mixture of neoavarol (**2**) (7 mg) and 10% palladium on carbon (10 mg) in methanol (2 ml) was vigorously stirred under a hydrogen atmosphere at room temperature for 20 min. The

reaction mixture was filtered through a celite column, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ether = 5 : 1 as an eluent) to give 4,11-dihydroneoavarol (**7**) (3 mg). The diastereo isomer of **7** was not detected.

4,11-Dihydroneoavarol (7) Colorless crystals. mp 110—112 °C. $[\alpha]_D$ −11.6° (c = 0.16, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3260, 1503, 1402, 1189. ^{1}H-NMR ($CDCl_3$) δ: 0.66 (3H, d, J = 6.7 Hz, H-11), 0.79 (3H, s, H-12), 0.82 (3H, s, H-14), 0.98 (3H, d, J = 6.3 Hz, H-13), 2.53 (1H, d, J = 14.3 Hz, H-15), 2.59 (1H, d, J = 14.3 Hz, H-15), 6.56—6.61 (3H, m, H-3′, -4′, -6′). EI-MS m/z: 316 (M^+). High-resolution MS Calcd for $C_{21}H_{32}O_2$: 316.2399. Found: 316.2398.

Catalytic Hydrogenation of 1 Avarol (**1**) (10 mg) was hydrogenated under conditions similar to those used for the hydrogenation of **2**, to give 3,4-dihydroavarol (4 mg), $[\alpha]_D$ −12.9° (c = 0.14, $CHCl_3$), which was identical with 4,11-dihydroneoavarol (**7**).

Oxidation of 2 with Silver(I) Oxide A mixture of neoavarol (**2**) (5 mg) and silver(I) oxide (29 mg) in dry ether was stirred at room temperature for 15 min. The reaction mixture was filtered through a Celite column, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ether = 4 : 1 as an eluent) to give neoavarone (**4**) (4 mg).

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References and Notes

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