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; neaavaro; neoavaron; 4'-methoxyavaron; 4'-methoxyneavaron

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 neaavaron. 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)5) (18 mg), which was also isolated from D. avara, and 4 (2 mg) named neoavaron. 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'-methoxyavaron and 4'-methoxy-neaavaron, respectively. Compounds 1 and 2 showed ichthyotoxic activity against killifish, Oryzias latipes (minimum lethal concentration 20 μg/ml for each compound).

Structures of Neaavaron (2) and Neoavaron (4) Neoavaron (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 (1H-NMR) (Table I) and carbon-13 nuclear magnetic resonance (13C-NMR) spectra of 2 showed the signals due to three methyl groups [1H-NMR δ: 0.86 (3H, s), 1.00 (3H, d, J=6.1 Hz), 1.06 (3H, s)], a benzylmethylene group [1H-NMR δ: 2.51 (1H, d, J=14.3 Hz), 2.63 (1H, d, J=...

Table I. 1H-NMR Data for 1—6 (400 MHz, CDCl3, J in Hz)

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<tr>
<th>Proton</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>3</td>
<td>5.14 (1H, brs)</td>
<td>5.13 (1H, brs)</td>
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<td>1.53 (3H, s)</td>
<td>4.45 (1H, brs)</td>
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<td>11</td>
<td>1.51 (3H, d, J=1.4)</td>
<td>1.53 (3H, brs)</td>
<td>1.53 (3H, s)</td>
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<td>1.06 (3H, s)</td>
<td>1.00 (3H, s)</td>
<td>1.05 (3H, s)</td>
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<tr>
<td>13</td>
<td>1.00 (3H, d, J=6.1)</td>
<td>0.93 (3H, d, J=6.6)</td>
<td>0.93 (3H, d, J=6.5)</td>
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<td>14</td>
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<td>2.44 (1H, d, J=13.4)</td>
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<td>2.63 (1H, d, J=14.3)</td>
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<td>6.59 (1H, d, J=7.2)</td>
<td>6.75 (1H, d, J=10.1)</td>
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<td>18</td>
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<td>6.70 (1H, dd, J=2.4)</td>
<td>6.70 (1H, dd, J=2.4, 8.5)</td>
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<td>6.45 (1H, d, J=2.4)</td>
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<td>21</td>
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<td>3.61 (3H, s)</td>
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14.3 Hz)], an exomethylene group [1H-NMR δ: 4.39 (1H, br s), 4.43 (1H, br s); 13C-NMR δ: 102.9 (t)], and a phenolic group [1H-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 neoavaron 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, [α]D = -11.6° (c = 0.16, CHCl3). 1H-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, [α]D = -12.9° (c = 0.16, CHCl3). From this finding the structure of neoavaron was elucidated to be 2, and the absolute structure of neoavaron 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 neoavaron (4) [molecular weight 312, IR 1685 cm⁻¹, UV 247 nm (ε 14900)] showed the presence of a conjugated enone moiety. The 1H-NMR data of 4 are closely related to those of neoavaron (2) as shown in Table I, except for the chemical shift and coupling constants of the olefinic proton signals [δ: 6.45 (1H, d, J = 2.4 Hz), 6.70 (1H, dd, J = 2.4, 10 Hz)] and 6.75 (1H, d, J = 10 Hz)]. These findings suggested that 4 is the quinone of neoavaron (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 neoavaron was elucidated to be 4.

Structures of 4'-Methoxyavaron (5) and 4'-Methoxy-

neoaavaron (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 1H-NMR spectrum of 5 was very similar to that of neoavaron (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'-methoxyavaron. Similarly the structure of 6 (molecular weight 342) was assigned as 4'-methoxyneoaavaron on the basis of spectroscopic analysis involving the comparison of the spectral data with those of neoavaron (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 in natural products from sources other than marine sponges. Sponge-derived sesquiterpenoid hydroquinones and quinones of this type, such as avarol,6 areolar,7 and smenospongic acid8 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. 1H- and 13C-NMR spectra were recorded with a Bruker AM-400 spectrometer (400 MHz for 1H and 100 MHz for 13C). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (δ singlet; d, doublet; t, triplet; 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 Davision Silica gel BW-820MH (70–200 mesh). HPLC was conducted with a YMC HPLC-8502 apparatus using YMC-Pack D-ODS-5 (octadecyl-silica 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 2d. The methanol extract (13.5 g) was suspended in water and extracted with a mixture of ethyl chloride and ethyl acetate (1:1). The methylene chloride-ethyl acetate-soluble portion (2.7 g) which showed a chiroptihyony activity as well as killing (40 mg in 5 ml) 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 : H2O = 4:1 as an eluent) to give avarol (1) (363 mg) and neoavaron (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% AgNO3-impregnated silica gel column chromatography (hexane:ether = 30:1 as an eluent) to give avaron (3) (18 mg) and neoavaron (4) (2 mg). From the 3rd fraction (103 mg, eluted with hexane:ethyl acetate = 2:1), 4'-methoxyavaron (5) (5 mg) and 4'-methoxyneoaavaron (6) (7 mg) were isolated by silica gel column chromatography (hexane:ether = 4:1 as an eluent) followed by HPLC (methanol : H2O = 10:1 as an eluent).
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-dihydronorovarol (7) (3 mg). The diastereoisomer of 7 was not detected.

4,11-Dihydronorovarol (7) Colorless crystals. mp 110—112°C. [α]_D = -11.6° (c = 0.16, CHCl₃). IR νmax cm⁻¹: 3260, 1503, 1402, 1189. ¹H-NMR (CDCl₃) δ: 6.66 (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 C₁₂H₁₅O₃: 316.2399. Found: 316.2398.

Catalytic Hydrogenation of Avarol (1) (10 mg) was hydrogenated under conditions similar to those used for the hydrogenation of 2, to give 3,4-dihydroavrarol (4 mg), [α]_D = -12.9° (c = 0.14, CHCl₃), which was identical with 4,11-dihydronorovarol (7).

Oxidation of 2 with Silver(I) Oxide A mixture of neovarol (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 neovarone (4) (4 mg).

Acknowledgement We thank Dr. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam, for the identification of the sponge.

References and Notes
3) The sponge was identified by Dr. R. W. M. van Soest, University of Amsterdam, who informed us that the color (but not the surface characters) of the sponge is similar to that of Dysidea herbacea.