# Two New Hydroquinone Derivatives from Two Sponge Species of the Aegean Sea

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**Summary**:From two sponge species (*Spongia and Ircinia*) of the Aegean Sea two new octaprenylated hydroquinone, 1,4,44-trihydroxy-2-octaprenylbenzene (1) and 4-hydroxy-3-octaprenylbenzoic acid (2) were isolated together with eleven known terpenoids.

The structures of the compounds were decided by using 1D- and 2D-NMR techniques. They were also tested against two gram-positive bacteria strains. Compound (1) exhibited weak activity against *Pseudomonas aeuroginosa*.

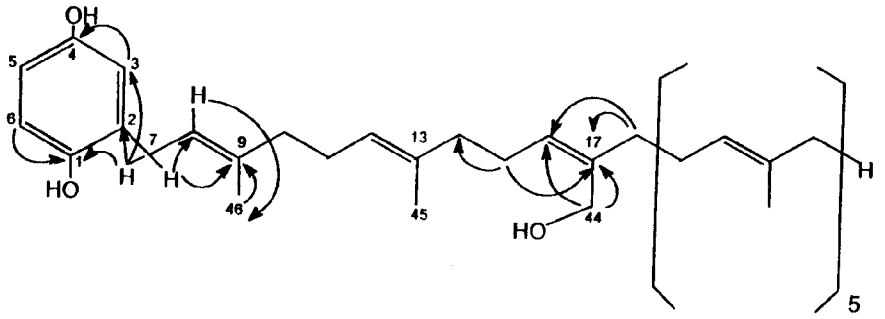
## Introduction

Marine sponges contain large amounts of prenylated aromatic compounds of a benzenoid nature which possess some biological activities such as analgesic [1], antimicrobial [2,3], antioxidant [4], ichtyotoxic [5,6] and cytotoxic [7-11]. In order to investigate the bioactive metabolites from marine organisms of the Aegean Sea, two sponge species were collected from the west coast of Turkey. During isolation and structure determination studies, eleven known terpenoids along with two new compounds have been obtained [12].

In the present study, the isolation and structural elucidation of two new hydroquinone derivatives (1) and (2) were reported. Their structures were decided by using 1D and 2D NMR techniques.

## Results and Discussion

Seven known terpenoids, squalene, furospinulosin-1, furospongin-1, 2-(hexaprenylmethyl)-2-methylchromenol, heptaprenylated *p*-quinol, 12-*epi*-




**Compound 1:** Arrows indicate selected HMBC correlations

deoxoscalarin and a mixture of furospongin-3- and 4 were isolated along with compound (1) from *Spongia officinalis.*

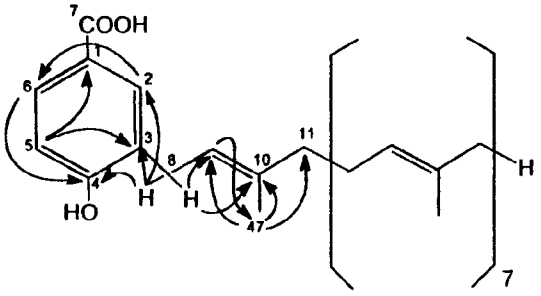
Compound 1 was obtained as a yellow oil from the first sponge which was light-brown in color. IR spectrum showed a hydroxyl absorption band at 3360 $cm^{-1}$. $^1H$ and $^{13}C$-NMR had diagnostic signals for an isoprenyl side chain including eight vinyl methyls, incorporating an all-trans geometry. Three aromatic proton signals were indicative of a 1,2,4-trisubstituted hydroquinone which was confirmed by a prominent base peak at m/z 161 in EIMS. A 2H singlet at 4.12 ppm in $^1H$-NMR was assigned to be the primary hydroxyl group. It was placed at C-44 by HMBC correlation (Table-1). The length of the isoprenyl side chain was deduced by a molecular ion at m/z 671 $[M+H]^+$, corresponding to the molecular formula $C_{46}H_{70}O_3$ and the structure of compound (1) was found to be 1,4,44-trihydroxy-2-octaprenylbenzene. The spectral data agreed well with that of related compounds [13,14].

Four known terpenoids, 4-hydroxy-3-tetraprenylphenyl acetic acid, demethyl-furospongin-4, dorisenone D, and 11β-acetoxyspongi-12-en-16-one were isolated along with compound (2) from *Ircinia spimulosa.*

Compound 2 was obtained as a yellow oil from the second sponge which was dark-brown in color. Two aromatic signals were observed in the $^1H$-NMR spectrum. The signal at 7.88 ppm was assigned to deshielded protons *ortho* to a carboxyl whose carbonyl group resonated at 170.6 ppm in the $^{13}C$-NMR spectrum. The IR spectrum of compound 2 pointed to the existence of a carboxyl group with the broad absorption at 2900 $cm^{-1}$. The signals attributable for a polyprenyl side chain were seen in the $^{13}C$-NMR spectrum. The chemical shifts of

Table-1: NMR data for compound (1)

| C | $^{13}$C-NMR | $^1$H-NMR | COSY | HMBC |
|---|---|---|---|---|
| 1 | 148.1s | | | |
| 2 | 116.4d | 6.65(d, 8.0) | H3 | C4,C6 |
| 3 | 113.7d | 6.55(dd, 3.0, 8.0) | H2 | |
| 4 | 149.4s | | | |
| 5 | 116.5d | 6.59(d,3.5Hz) | | C1 |
| 6 | 128.4s | | | |
| 7 | 29.6t | 3.30(d,7.0) | H8 | C1,C5,C6,C8,C9 |
| 8 | 121.5d | 5.28-5.30 m | H7,H10, C10,C46 | |
| 9 | 138.4s | | | |
| 10 | 39.9t | 1.96-2.01 m | | C8,C9,C11,C46 |
| 11 | 26.7t | 2.04-2.09 m | H12 | C10,C12, C13, C45 |
| 12 | 124.2d | 5.09-5.13 m | H11 | C14, C45 |
| 13 | 134.8s | | | |
| 14 | 39.7t | 1.96-2.01 m | | C12, C13, C15, C45 |
| 15 | 26.6t | 2.12-2.18 m | H16 | C14, C16, C17 C44 |
| 16 | 128.9d | 5.28-5.30 m | H15, H18 | |
| 17 | 138.3s | | | |
| 18 | 35.3t | 2.12-2.18 m | H16 | C16, C17, C19 |
| 19 | 26.2t | 2.04-2.09 m | H20 | C18, C20, C21, C43 |
| 20 | 123.7d | 5.09-5.13 m | H-19 | C21, C43 |
| 21 | 134.9s | | | |
| 22 | 39.6t | 1.96-2.01 m | | C20,C21,C23 |
| 23 | 27.0t | 2.04-2.09 m | H24 | C22, C24, C25, C42 |
| 24 | 124.9d | 5.09-5.13 m | H23 | C25, C42 |
| 25 | 134.4s | | | |
| 26 | 39.8t | 1.96-2.01 m | | C25, C27, C28 |
| 27 | 26.4t | 2.04-2.09 m | H28 | C26, C28 C29, C41 |
| 28 | 124.1d | 5.09-5.13 m | H27 | C29,C41 |
| 29 | 138.2s | | | |
| 30 | 39.6t | 1.96-2.01 m | | C28, C29, C31 |
| 31 | 26.6t | 2.04-2.09 m | H32 | C30, C32, C33, C40 |
| 32 | 123.9d | 5.09-5.13 m | H31 | C33, C40 |
| 33 | 128.2s | | | |
| 34 | 39.7t | 1.96-2.01 m | | C33, C35 |
| 35 | 26.2t | 2.04-2.09 m | H36 | C34, C36, C37 |
| 36 | 124.4d | 5.09-5.13 m | H27 | C30, C41 |
| 37 | 135.5s | | | |
| 38 | 25.7q | 1.68s | H36 | C36,C37,C39 |
| 39 | 17.7q | 1.59s | | C36,C37,C38 |
| 40 | 16.0q | 1.59s | H32 | C31,C32,C33 |
| 41 | 16.1q | 1.59s | H28 | C28,C29,C30 |
| 42 | 16.1q | 1.59s | H24 | C23,C24,C25 |
| 43 | 16.0q | 1.59s | H20 | C20,C21, C22 |
| 44 | 60.4t | 4.12s | | C16, C17 |
| 45 | 16.1q | 1.59s | H12 | C12,C13, C14 |
| 46 | 16.0q | 1.59s | | C9, C10 |




**Compund 2:** Arrows indicate selected HMBC correlations

Table-2: NMR data for compound (2)

| C | $^{13}$C-NMR | $^{1}$H-NMR | COSY | HMBC |
|---|---|---|---|---|
| 1 | 121.5s | | | |
| 2 | 132.5d | 7.87m | | C6,C8 |
| 3 | 126.7s | | | |
| 4 | 159.5s | | | |
| 5 | 115.8d | 6.85 (d, 9.0) | | C1, C3 |
| 6 | 130.5d | 7.88m | | C2, C4 |
| 7 | 170.6s | | | |
| 8 | 29.8t | 3.41(d, 7.0) | | C2, C3, C4, C9 |
| 9 | 120.7d | 5.32(t, 7.5) | | C47 |
| 10 | 139.6s | | | |
| 11 | 39.8t | 1.97-2.00 m | | C12, C13 |
| 12 | 26.8t | 2.05-2.13 m | | C11, C13 C14 |
| 13 | 123.4d | 5.11 m | H11, H12 | C11, C12 |
| 14 | 135.7s | | | |
| 15 | 39.7t | 1.97-2.00 m | H46 | C14, C16, C17, C46 |
| 16 | 26.7t | 2.05-2.13 m | | C15, C17, C18, C45 |
| 17 | 124.4d | 5.11 m | H15, H16 | C16, C19, C45 |
| 18 | 135.0s | | | |
| 19 | 39.7t | 1.97-2.00 m | H45 | C20, C21, C45 |
| 20 | 26.6t | 2.05-2.13 m | | C19, C21, C22, C45 |
| 21 | 124.3d | 5.11 m | H19, H20 | C19, C20, C44 |
| 22 | 135.0s | | | |
| 23 | 39.7t | 1.97-2.00 m | H44 | C24, C25, C44 |
| 24 | 26.6t | 2.05-2.13 m | | C23, C25, C26, C43 |
| 25 | 124.3d | 5.11 m | H23, H24 | C23, C24, C43 |
| 26 | 134.9s | | | |
| 27 | 39.6t | 1.97, 2.00 m | H43 | C28, C29, C43 |
| 28 | 26.6t | 2.05-2.13 m | | C27,C29,C39, C42 |
| 29 | 124.3d | 5.11 m | H27, H28 | C27, C28, C42 |
| 30 | 134.9s | | | |
| 31 | 39.6t | 1.97-2.00 m | H42 | C32, C33, C42 |
| 32 | 26.6t | 2.05-2.13 m | | C31, C33, C34, C41 |
| 33 | 124.2d | 5.11 m | H31, H32 | C31, C32, C41 |
| 34 | 134.9s | | | |
| 35 | 39.6t | 1.97-2.00 m | | C36, C37, C41 |
| 36 | 26.3t | 2.05-2.13 m | | C35, C37, C38, C40 |
| 37 | 124.2d | 5.11 m | H35, H36 | |
| 38 | 131.2s | | | |
| 39 | 25.7q | 1.67s | | C37, C38, C40 |
| 40 | 16.0q | 1.59s | | C37, C38, C39 |
| 41 | 16.1q | 1.59s | H35 | C33, C34, C35 |
| 42 | 16.0q | 1.59s | H31 | C29, C30, C31 |
| 43 | 16.0q | 1.59s | H27 | C25,C26, C27 |
| 44 | 16.0q | 1.59s | H23 | C21, C22, C23 |
| 45 | 16.0q | 1.59s | H19 | C17, C18, C19 |
| 46 | 16.0q | 1.59s | H15 | C13, C14, C15 |
| 47 | 16.3q | 1.79s | H11, H12 | C9, C10, C12 |

methyl groups indicated all-*trans* configuration for the double bonds (Table-2). A molecular ion at m/z 682 in EIMS suggested a molecular composition for compound 2 as $C_{47}H_{70}O_3$. The spectral data were in accordance with the data reported for 4-hydroxy-3-tetraprenyl benzoic acid [15], and the structure of compound 2 was elucidated to be 4-hydroxy-3-octaprenylbenzoic acid.

The new compounds, (1) and (2), were analyzed for antibacterial activity against gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus*, and gram-negative *Pseudomonas aeuroginosa* and *Escherichia coli*. Compound (1) (3.0 mg) was purely isolated from the sixth fraction of the first sponge by normal phase HPLC eluted with heptane-EtOAc-$CH_2Cl_2$ (6:2:1) along with seven known terpenoids. The second fraction from the second sponge was fractionated on silica gel to give three subfractions. Compound 2 (5.3 mg) was obtained from the third subfraction using normal phase HPLC eluted with heptane-EtOAc (3:2) in addition to four known terpenoids.

Antibacterial activity was assessed by using the standard agar-plate zone of inhibition method at the concentration of 0.5 mg/disk. While compound (2) did not show activity against any of bacterium strains, however, compound (1) exhibited weak activity against *Pseudomonas aeroginosa* causing an inhibition zone of 10 mm in diameter (The diameter of paper disks used was 8 mm).

## Experimental

### *Instrumentation*

$^{1}$H-NMR spectra were run at 500 MHz and $^{13}$C-NMR spectra at 125 MHz in $CDCl_3$, using JEOL $\alpha$ 500 (500 MHz) FT NMR spectrometer. Chemical shifts are given in ppm relative to the international standard of TMS. Methyl, methylene and methine carbons were distinguished by DEPT experiments. One bond heteronuclear $^{1}$H - $^{13}$C connectivities were determined with 2D HMQC experiments. EIMS spectra were recorded on a Hitachi M-2500 instrument. FT-IR spectra were taken on a Jasco FT/IR-300 infrared spectrophotometer in $CCl_4$. HPLC separations were carried out on a Hitachi L-6000 apparatus equipped with a Hitachi L-4000 UV detector. All analytical thin layer chromatography

was performed on TLC plates precoated with Kieselgel 60 $F_{254}$ (Merck).

*Animal material*

One of the sponge species, dark brown in color was identified as *Spongia officinalis* and the other one, light-brown in color, was determined as *Ircinia spinulosa*. The sponges were collected from the west cost of Turkey, at the depth of 3 m, in August, 1996. The voucher samples of each sponge were kept at the Department of Biology, Faculty of Education, Gaza University, Ankara.

*Extraction and isolation*

Fresh samples of the sponges were cut into small pieces and extracted with ethanol three times at the room temperature. The extracts were concentrated under reduced pressure and the resulting aqueous residue of each sponge was partitioned between EtOAc and $H_2O$. Separation of the EtOAc-soluble materials was achieved initially by vacuum flash chromatography on Si 60 and then silica gel column. Seven and five fractions were obtained from the first and second sponges, respectively. Compound **1** (3.0 mg) was isolated in pure form from the sixth fraction of the first sponge by normal phase HPLC eluted with heptane-EtOAc-$CH_2Cl_2$ (6:2:1). The second fraction from the second sponge was subjected on silica gel to give three subfractions. Compound **2** (5.3 mg) was obtained from the third subfraction using normal phase HPLC eluted with heptane-EtOAc (3:2).

*1,4,44-Trihydroxy-2-octaprenylbenzene* (**1**)

Yellow oil; IR $\nu_{max}^{film}$ $cm^{-1}$; 3350, 2900, 980; EIMS (70eV) m/z (Rel.int.): 671 (17) $[M+H]^+$, 653 (50) $[M-H_2O]^+$, 584(14), 516(23), 447(15), 339(21), 271(15), 161(82), 123(69), 69(100); $^1$H-NMR ($CDCl_3$): δ 6.65 (1H, d, H-2), 6.59(1H, d, 3.5, H-5), 6.55 (1H, dd, 3.0, 8.0, H-3), 5.28-5.30(2H, m, H-8, H-16), 5.09-5.13 (6H, m, H-12, H-20, H-24, H-28, H-32, H-36), 4.12 (2H, s, H-44), 3.30(2H, d, 7.0, H-7), 2.12-2.18 (4H, m, H-15, H-18), 2.04-2.09(12H, M, H-11, H-19, H-23, H-27, H-31, H-35), 1.96-2.01 (12 H, m, H-10, H-14, H-22, H-26, H-30, H-34), 1.68(3H, s, H-38), 1.59(21H, s, H-39, H-40, H-41, H-42, H-43, H-45, H-46); $^{13}$C-NMR ($CDCl_3$): δ 149.4 (s, C-4), 148.1 (s, C-1), 138.4(s, C-9), 138.3(s, C-17), 138.1(s, C-29), 135.5(s, C-37), 134.9(s, C-21), 134.8(s, C-13), 134.4(s, C-25), 128.9(d, C-16), 128.4(s, C-6), 128.2(s, C-33), 124.9(d, C-24), 124(d, C-36), 124.2(d, C-12), 124.1(d, C-28), 123.9(d, C-32), 123.7(d, C-20), 121.5(d, C-8), 116.5(d, C-5), 116.4(d, C-2), 113.7(d, C-3), 60.4(t, C-44), 39.9 (t, C-10), 39.8(t, C-26), 39.7(t, C-14, C-34), 39.6(t, C-22, C-30), 35.2(t, C-18), 29.6(t, C-7), 27.0(t, C-23), 26.7(t, C-11), 26.6 (t, C-15, C-31), 26.4(t, C-27), 26.2(t, C-19, C-35), 25.7 (q, C-38), 17.7(q, C-39), 16.1 (q, C-41, C-42, C-45), 16.0 (q, C-40, C-43, C-46).

*4-Hydroxy-3-octaprenylbenzoic acid* (**2**)

Yellow oil; IR $\nu_{max}^{film}$ $cm^{-1}$; 2900, 1700, 1400, 960, 840, 720; EIMS (70eV) m/z (Rel.int.); 682 (10) $[M^+]$, 638(20), 571(12), 531(17), 477(20), 409(25), 297(5), 203(28), 161(66), 135(61), 107(65), 68(100); $^1$H-NMR ($CDCl_3$: δ 7.88 (1H, m, H-6), 7.87(1H, m, H-2), 6.85(1H, d, 9.0, H-5), 5.32(1H, t, 7.5, H-9), 5.11 (7H, m, H-13, H-17, H-21, H-25, H-29, H-33, H-37), 3.41(2H, d, 7.0 H-8), 2.05-2.13(14H, m, H-12, H-16, H-20, H-24, H-28, H-32, H-36), 1.97-2.00(14H, m, H-11, H-15, H-19, H-23, H-27, H-31, H-35), 1.79 (3H, s, H-47), 1.67 (3H, s, H-39), 1.59(21H, s, H-40, H-41, H-42, H-43, H-44, H-45, H-46), $^{13}$C-NMR ($CDCl_3$): δ 170.6(s, C-7), 159.5(s, C-4), 139.6(s, C-10), 135.7(s, C-14), 135.0(s, C-18), 134.9(s, C-26), C-30, C-34), 132.5(d, C-2), 131.2(s, C-38), 130.5(d, C-6), 126.7(s, C-3), 124.4(d, C-17), 124.3(d, C-21, C25, C-29), 124.2(d, C-33, C-37), 123.4(d, C-13), 121.5(s, C-1), 120.7(d, C-9), 115.8(d, C-5), 39.8(t, C-11), 39.7(t, C-15, C-19, C-23), 39.6(t, C-27, C-31, C-35), 29.8(t, C-8), 26.8(t, C-12), 26.7(t, C-16), 26.6(t, C-20, C-24, C-28, C-32), 26.3(t, C-36), 25.7(q, C-39), 16.3(q, C-47), 16.1(q, C-41), 16.0(q, C-40, C-42, C-43, C-44, C-45, C-46).

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**References**

1. M. Ochi, H. Kotsuki, S. Inoue, M. Taniguchi, T. Tokoroyama, *Chem. Lett.*, 331, (1979).
2. F.J. Mc Enroe, W. Fenical, *Tetrahedron*, **34**, 1661 (1978).
3. G. Cimino, S. De Stefano, L. Minale, *Experientia*, **28**, 1401 (1972).

4. A. Sato, T. Shindo, N. Kasanuki, K. Hasegawa, *J. Nat. Prod.*, **52**, 975 (1989).
5. T. Higa, J. Tanaka, A. Kitamura, T. Koyama, M. Takakashi, T. Uchida, *Pure and Appl. Chem.*, **66**, 2227 (1994).
6. M.N. Dave, T. Kusumi, M. Ishitsuka, T. Iwashita, H. Kakisawa, *Heterocycles*, **22**, 2301 (1984).
7. B.M. Howard, K. Clarckson, R.L. Bernstein, *Tetrahedron Lett.*, **21**, 4449 (1979).
8. H.H. Sun, N.M. Ferrara, O.J. Mc Connell, W. Fenical, *Tetrahedron Lett.*, **21**, 3123 (1980).
9. F.J. Schmitz, W. Lakshmi, D.R. Powell, D. Van Der Helm, *J. Org. Chem.*, **49**, 241 (1984).
10. N. Fusetani, K. Yasukawa, S. Matsunaga, K. Hashimoto, *Tetrahedron Lett.*, **26**, 6449 (1985).
11. G.J. Hooper, M.T. Davies-Coleman, *Tetrahedron Lett.*, **36**, 3265 (1995).
12. I. Erdogan, "Studies on the Chemical Constituents of Two Sponges from the Aegean Sea and of the Okinawan Sponge *Theonella cupola*", Master Thesis, University of the Ryukyus, Graduate School of Department of Biology, Chemistry and Marine Sciences, Okinawa, Japan (1998).
13. Y.F. Pouchus, J.F. Verbist, J.F. Baird, K. Boukef, *J. Nat. Prod.*, **51**, 188 (1988).
14. P. Rivera, F. Podesta, M. Norte, F. Cataldo, A.G. Gonzales, *Can. J. Chem.*, **68**, 1399 (1990).
15. G. Cimino, S. De Stefano, L. Minale, *Experientia*, **28**, 1401 (1972).