Antibacterial and Cytotoxic Natural and Synthesized Hydroquinones from Sponge *Ircinia spinosula*

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In order to check the structure-activity relationship and find more potent derivatives of the natural products **1** and **2** obtained from sponge *Ircinia spinosula*, a series of oxidation, hydrogenation, acetylation and methylation derivatives was prepared. All compounds (natural and synthetic ones) were screened for their cytotoxic and antibacterial activities. The biological studies showed a wide range of antibacterial activity even though only **2** and **2d** showed a moderate cytotoxicity against the clone C98. The oxidation of the hydroquinone to quinone and the hydrogenation of the side-chain increased the antibacterial effect of the molecules.

Introduction

Several naturally occurring prenyl hydroquinones with a terpenoid portion that ranges from one to eight isoprene units have been described (Cimino *et al.*, 1972). Triterpenyl- and tetraterpenyl- hydroquinones are the most abundant compounds among this class of metabolites (Cimino *et al.*, 1972). Biological activities have been reported for relatively few polyprenyl-hydroquinones (Higa, 1991). *Ircinia spinosula* is a black massive sponge usually found in shallow Mediterranean marine ecosystems. Previous studies on the organism report several 2-polyprenylbenzoquinones, 2-polyprenylbenzoquinols, prenylated furans and a C-31 difuranoterpene (Cimino *et al.*, 1972; Minale, 1978) along with their activities on phospholipase A_2, the analgesic, muscle relaxant and the anti-inflammatory effects (De Pasquale *et al.*, 1991; Gil *et al.*, 1995). Their biological effects in antimicrobial, brine shimp, and fish lethality assays have also been reported (De Rosa *et al.*, 1994) showing moderate activities.

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In the framework of investigations towards the discovery of Mediterranean marine organisms with pharmacological interest (Teeyapant *et al.*, 1993; Köning and Wright, 1996), we report in this study the chemical constituents obtained from *I. spinosula* either by isolation (**1** and **2**) or by synthesis (eleven synthetic derivatives, **1a**–**g** and **2a**–**d**) and the determination of their structure-activity relationships in cytotoxic and antibacterial assays.

Materials and Methods

General details

IR spectra were obtained using a FT-IR NICOLET mod. IMPACT 420NI-912 AO354 infrared spectrophotometer. UV was recorded on a SHIMADZU UV model 160A. ^{1}H and ^{13}C-NMR spectra were recorded using a BRUKER AC 200 spectrometer. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Mass spectral data were recorded on a HEWLETT PACKARD 5973 and a FINNIGAN TSQ 7000 Mass Selective Detector. Column chromatography was performed with Kieselgel 60 (Merck).

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TLC were performed with Kieselgel 60 F_{254} (Merck 5554).

Sponge material

Sponge *Ircinia spinosula* was collected by SCUBA (2–15 meters) from Saronicos Gulf, Greece and kept deep frozen. A voucher specimen is deposited at the Herbarium of the Laboratory of Pharmacognosy (ATPH/MO/35).

Extraction and isolation

The organism was initially freeze dried (dry weight 673.0 g) and then exhaustively extracted at room temperature with mixtures of CH_2Cl_2/MeOH (3/1, v/v). The organic extract after evaporation of the solvents afforded a dark green oily residue (30.1 g) which was subjected to vacuum column chromatography using silica gel and a gradient solvent system ranging from 100% CH_2Cl_2 to 100% EtOAc. The polarity of the eluents was increased in increments of 2% EtOAc. The CH_2Cl_2/EtOAc (98/2–94/6) fractions were pooled together and further subjected to vacuum column chromatography using a gradient solvent system with cyclohexane and EtOAc to afford pure metabolite **1** (6.61 g). The same process was repeated for the CH_2Cl_2/EtOAc (90/10–84/16) fractions to yield metabolite **2** (5.04 g) in pure state. Structural elucidation of natural products **1** and **2** was based on comparison of their spectral data with those of the literature (Cimino *et al.*, 1972). The structures of the products in all cases are supported by their spectral characteristics.

Syntheses of analogues

The eleven hydroquinone derivatives were prepared by simple chemical manipulations, such as oxidation, acetylation, permethylation of the hydroxyl groups and hydrogenation of the double bonds (Fig. 1). Polyprenylated hydroquinones **1** and **2** were treated with AcOAc in dry pyridine to afford the corresponding acetylated products **1a** and **2a**. Reduction of these products with H_2 produced the saturated analogues **1b** and **2b**. Oxidation with CrO_3 in 70% acetic acid solution led to the conversion of natural product **1** to quinone **1d** and natural product **2** to derivatives **2c** and **2d**. Permethylation and reduction of metabolite **1** gave compounds **1c** and **1f**. The analogue **1e** was afforded by oxidation of compound **1f**. Finally hydroquinone **1** was treated with PFBCl in CH_3CN to afford **1 g**.

Spectral data

2-octaprenyl-1,4-hydroquinone (**1**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ=6.51–6.67 (3H, m), 5.28 (1H, t, J=7.9Hz), 5.08 (7H, m), 3.29 (2H, d, J=7.1Hz), 1.91–2.12 (28H, m), 1.73 (3H, s), 1.68 (3H, s), 1.49 (21H, brs); ^{13}C-NMR ($CDCl_3$, 200MHz) δ=149.3 (s), 147.9 (s), 138.3 (s), 135.4 (s), 134.9 (s), 134.8 (s, x3), 131.1 (s), 128.3 (s), 124.3 (d), 124.2 (d), 123.7 (d), 121.3 (d), 116.6 (d), 116.4 (d), 113.6 (d), 39.6 (t), 29.5 (t), 26.7 (t), 26.6 (t, x2), 26.4 (t), 25.6 (q), 17.6 (q), 16.1 (q), 16.0 (q), 15.9 (q). IR (film): ν_{max} = 3400, 2920, 1500, 1460, 1380, 1180 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 211 (10400), 291 (2600) nm; EI-MS: *m/z* (% rel. int.) = 654 ([M] $^+$, 5), 586 (3), 246 (8), 203 (12), 177 (18), 161 (45), 123 (52), 69 (100).

2-octaprenyl-1,4-diacetoxy-benzene (**1a**): A volume of AcOAc (1 ml) was added to a solution of **1** (650.0 mg, 0.99 mmol) in pyridine (5 ml) and the mixture was stirred at 50° C overnight. In the resulting mixture a quantity of CH_2Cl_2 and H_2O was added and the organic layer was separated, washed with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **1a** (605.1 mg, 82.8%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.91–7.05 (3H, m), 5.25 (1H, t, J=8.0 Hz), 5.12 (7H, m), 3.24 (2H, d, J=7.1Hz), 2.30 (3H, s), 2.28 (3H, s), 1.93–2.12 (28H, m), 1.70 (3H, s), 1.57 (24H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 168.8 (s), 168.7 (s), 148.0 (s), 146.1 (s), 137.2 (s), 134.8 (s), 134.6 (s), 134.5 (s), 130.8 (s), 124.2 (d), 124.1 (d), 123.8 (d), 122.6 (d), 122.4 (d), 120.5 (d), 119.6 (d), 39.5 (t), 28.3 (t), 26.5 (t), 26.4 (t), 26.3 (t), 25.4 (q), 20.7 (q), 20.4 (q), 17.4 (q), 15.9 (q), 15.8 (q); IR (film): ν_{max} = 2920, 1770, 1490, 1440, 1370, 1200, 1180 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 227 (26400), 266 (4200) nm; EI-MS: *m/z* (% rel. int.) = 738 ([M]$^+$, 2), 670 (15), 601 (13), 533 (18), 465 (24), 397 (27), 189 (83), 43 (100); *Anal.* Calcd for $C_{50}H_{74}O_4$: C, 81.24; H, 10.10. Found: C, 81.27; H, 10.16.

2-octaisopentyl-1,4-diacetoxy-benzene (**1b**): A solution of **1a** (85.7 mg, 0.11 mmol) in EtOH

(10 ml) was hydrogenated using 10% Pd/C under an atmosphere of H_2. The mixture was stirred at 50° C overnight. Then the catalyst was removed by filtration and the solvent was evaporated to give pure **1b** (82.5 mg, 99.09%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.85–7.03 (3H, m), 2.41–2.55 (2H, m), 2.29 (3H, s), 2.26 (3H, s), 1.05–1.43 (52H, m), 0.85 (3H, d, *J*=6.5Hz), 0.84 (21H, brs), 0.83 (3H, d, *J*=6.5Hz); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 169.3 (s), 148.2 (s), 146.2 (s), 136.2 (s), 123.0 (d), 122.8 (d), 119.7 (d), 39.4 (t), 37.4 (t), 37.2 (t), 32.8 (d), 27.9 (d), 27.8 (t), 24.8 (t), 24.5 (t), 22.7 (q), 22.6 (q), 21.1 (q), 20.8 (q), 19.7 (q), 19.5 (q); IR (film): ν_{max} = 2920, 1765, 1490, 1460, 1370, 1200, 1170 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 201 (11600), 267 (550) nm; EI-MS: *m/z* (% rel. int.) = 754 ([M]$^+$, 2), 712 (3), 671 (15), 600 (28), 195 (8), 149 (18), 122 (32), 86 (95), 49 (100); *Anal.* Calcd for $C_{50}H_{90}O_4$: C, 79.50; H, 12.02. Found: C, 79.42; H, 12.00.

2-octaprenyl-1,4-dimethoxy-benzene (**1c**): K_2CO_3 (300 mg, 2.1 mmol) was added to a solution of **1** (200.0 mg, 0.30 mmol) in dry acetone (20 ml) and the mixture was stirred at room temperature for 20 minutes. After addition of dimethylsulfate (1 ml, 10.5 mmol) the resulting mixture was refluxed overnight. Then a quantity of CH_2Cl_2 and H_2O was added and the organic layer was washed sequentially with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **1c** (184.1 mg, 92.0%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.61–6.78 (3H, m), 5.25–5.31 (1H, m), 5.09 (7H, m), 3.76 (3H, s), 3.73 (3H, s), 3.28 (2H, d, *J*=7.1Hz), 1.88–2.12 (28H, m), 1.67 (3H, s), 1.66 (3H, s), 1.57 (21H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 153.5 (s), 151.6 (s), 136.5 (s), 135.1 (s), 134.9 (s), 131.4 (s), 131.2 (s), 124.4 (d), 124.2 (d), 124.1 (d), 122.0 (d), 115.9 (d), 111.1 (d), 110.4 (d), 56.0 (q), 55.6 (q), 39.7 (t), 31.9 (t), 30.3 (t), 29.7 (t), 29.1 (t), 28.2 (t), 26.7 (t), 26.6 (t), 25.7 (q), 22.7 (q) 17.7 (q), 16.1 (q), 16.0 (q), 14.1 (q); IR (film): ν_{max} = 2920, 1500, 1440, 1220 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 214 (6600), 289 (1100) nm; EI-MS: *m/z* (% rel. int.) = 682 ([M]$^+$, 2), 515 (3), 339 (3), 271 (5), 161 (8), 122 (12), 84 (70), 49 (100); *Anal.* Calcd for $C_{48}H_{74}O_2$: C, 84.39; H, 10.92. Found: C, 84.36; H, 10.91.

2-octaprenyl-1,4-quinone (**1d**): A quantity of **1** (166.0 mg, 0.25 mmol) in acetone (3 ml) was added to a solution of CrO_3 (75.0 mg, 0.75 mmol) in 70% HOAc (10 ml). The resulting mixture was stirred at 50° C overnight before a volume of CH_2Cl_2 and H_2O was added. The organic layer was separated, washed with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **1d** (142.7 mg, 88%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.72 (2H, m), 6.47 (1H, d, *J*=2.9Hz), 5.27 (1H, t, *J*=7.2Hz), 5.04 (7H, m), 3.06 (2H, d, *J*=7.2Hz), 1.93–2.10 (28H, m), 1.61 (3H, s), 1.55 (3H, s), 1.53 (21H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 187.8 (s), 187.5 (s), 140.1 (s), 136.6 (s), 136.2 (s), 135.4 (s), 134.9 (s), 134.8 (s), 132.2 (s), 131.1 (s), 124.3 (d), 124.2 (d), 124.1 (d), 123.6 (d), 117.5 (d), 39.7 (t), 39.6 (t), 27.3 (t), 26.7 (t), 26.6 (t, x2), 26.3 (t), 25.6 (q), 17.6 (q), 16.1 (q), 16.0 (q), 15.9 (q); IR (film): ν_{max} = 2920, 1660, 1600, 1450, 1380 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 273 (55) nm; EI-MS: *m/z* (% rel. int.) = 652 ([M]$^+$, 3), 365 (5), 326 (31), 298 (31), 284 (48), 241 (60), 157 (12), 84 (78), 49 (100); *Anal.* Calcd for $C_{46}H_{68}O_2$: C, 84.59; H, 10.50. Found: C, 84.55; H, 10.42.

2-octaisopentyl-1,4-quinone (**1e**): A quantity of **1f** (10.0 mg, 0.015 mmol) in acetone (1 ml) was added to a solution of CrO_3 (5 mg, 0.05 mmol) in 70% HOAc (5 ml). The resulting mixture was stirred at 50° C overnight before a volume of CH_2Cl_2 and H_2O was added. The organic layer was separated, washed with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **1e** (8.01 mg, 80.0%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.63–6.70 (2H, m), 6.54 (1H, d, *J*=2.9Hz), 2.36–2.41 (2H, t, *J*=9.2Hz), 1.04–1.52 (52H, m), 0.83 (3H, d, *J*=6.2Hz), 0.82 (21H, brs), 0.80 (3H, d, *J*=6.2Hz); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 187.8 (s), 187.5 (s), 150.1 (s), 136.8 (d), 136.1 (d), 132.2 (d), 39.3 (t), 37.3 (t), 37.0 (t), 34.9 (t), 32.7 (d), 29.6 (t), 27.9 (d), 26.6 (t), 24.7 (t), 24.4 (t), 22.7 (q), 22.6 (q), 19.7 (q); IR (film): ν_{max} = 2920, 1660, 1450, 1380 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 246 (2200) nm; EI-MS: *m/z* (% rel. int.) = 668 ([M]$^+$, 5), 600 (5), 220 (8), 205 (15), 124 (23), 83 (60), 57 (100); *Anal.* Calcd for $C_{46}H_{84}O_2$: C, 82.55; H, 12.66. Found: C, 82.63; H, 12.70.

2-octaisopentyl-1,4-hydroquinone (**1f**): A solution of **1** (92.0 mg, 0.14 mmol) in EtOH (10 ml)

was hydrogenated using 10% Pd/C under an atmosphere of H_2. The mixture was stirred at 50° C overnight. Then the catalyst was removed by filtration and the solvent was evaporated to afford pure **1f** (93.2 mg, 99.2%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.50–6.64 (3H, m), 2.47–2.54 (2H, t, *J*=8.2Hz), 1.09–1.57 (52H, m), 0.85 (3H, d, *J*=6.2Hz), 0.84 (21H, brs), 0.82 (3H, d, *J*=6.2Hz); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 149.3 (s), 147.2 (s), 130.4 (s), 116.7 (d), 115.9 (d), 113.1 (d), 39.3 (t), 37.5 (t), 37.4 (t), 37.3 (t), 37.1 (t), 36.9 (t), 32.8 (d), 27.9 (d), 27.6 (t), 24.8 (t), 24.5 (t), 22.7 (q), 22.6 (q), 19.7 (q, x2), 19.6 (q), 19.5 (q); IR (film): ν_{max} = 3300, 2930, 1500, 1470, 1380, 1180 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 290 (2200), 219 (3050) nm; EI-MS: *m/z* (% rel. int.) = 670 ([M]$^+$, 3), 600 (25), 530 (3), 163 (8), 123 (95), 57 (100); *Anal.* Calcd for $C_{46}H_{86}O_2$: C, 82.31; H, 4.77. Found: C, 82.27; H, 4.71.

2-octaprenyl-1,4-pentafluorobenzyloxy-benzene (**1g**): PFBCl (75.9 mg, 0.33 mmol) was added to a solution of **1** (110 mg, 0.17 mmol) in CH_3CN (10 ml). The mixture was stirred at 50° C overnight under a N_2 atmosphere before it was concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **1g** (127.12 mg, 71.7%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 7.11–7.20 (3H, m), 5.20 (1H, t, *J*=7.9Hz), 5.03 (7H, m), 3.27 (2H, d, *J*=7.1Hz), 1.92–2.08 (28H, m), 1.59 (3H, s), 1.57 (3H, s), 1.51 (21H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 157.2 (s), 148.2 (PFB-ring), 148.1 (s), 146.3 (PFB-ring), 146.2 (s), 143.2 (PFB-ring), 142.2 (PFB-ring), 140.4 (PFB-ring), 138.7 (s), 135.4 (s), 135.3 (s), 135.2 (PFB-ring), 135.0 (s), 134.9 (s), 131.2 (s), 124.3 (d), 124.2 (d), 124.1 (d), 123.8 (d), 122.9 (d), 122.7 (d), 119.9 (d), 119.7 (d), 39.7 (t), 28.2 (t), 26.7 (t), 26.6 (t), 25.6 (q), 17.6 (q), 16.2 (q), 15.9 (q); IR (film): ν_{max} = 2920, 1760, 1650, 1500, 1420, 1370, 1200, 1000 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 204 (270000), 268 (25.800) nm; EI-MS: *m/z* (% rel. int.) = 1042 ([M]$^+$, 1), 973 (10), 836 (7), 600 (14), 510 (8), 355 (12), 194 (95), 69 (100); *Anal.* Calcd for $C_{60}H_{68}O_4F_{10}$: C, 69.06; H, 6.57. Found: C, 69.01; H, 6.62.

2-[24-hydroxy]-octaprenyl-1,4-hydroquinone (**2**): ^{1}H-NMR ($CDCl_3$, 200MHz): δ = 6.55–6.65 (3H, m), 5.74 (1H, brs, OH), 5.34 (2H, m), 5.09 (6H, m), 4.12 (2H, s), 3.27 (2H, d, *J*=7.1Hz), 1.99–2.12 (28H, m), 1.72 (3H, s), 1.67 (3H, s), 1.58 (18H, brs); ^{13}C-NMR ($CDCl_3$, 200MHz): δ = 149.5 (s), 147.9 (s), 138.1 (s), 137.9 (s), 135.5 (s), 135.3 (s), 134.9 (s), 134.8 (s), 134.4 (s), 131.2 (s), 129.1 (d), 128.3 (s), 124.9 (d), 124.3 (d), 124.2 (d), 124.1 (d), 123.9 (d), 123.8 (d), 121.6 (d), 116.5 (d), 116.4 (d), 113.6 (d), 60.3 (t), 39.8 (t), 39.7 (t), 39.6 (t), 35.2 (t), 29.5 (t), 26.9 (t), 26.7 (t), 26.6 (t), 26.4 (t), 26.3 (t), 26.2 (t), 25.7 (q), 17.6 (q), 16.1 (q), 16.0 (q), 15.9 (q); IR (film): ν_{max} = 3360, 2920, 1500, 1450, 1380, 1200 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 275 (850), 203 (6800) nm; EI-MS: *m/z* (% rel. int.) = 670 ([M]$^+$, 1), 220 (15), 205 (18), 145 (12), 84 (60), 49 (100).

2-[24-acetoxy]-octaprenyl-1,4-diacetoxy-benzene (**2a**): A volume of AcOAc (1 ml) was added to a solution of **2** (217.0 mg, 0.32 mmol) in pyridine (5 ml) and the mixture was stirred at 50° C overnight. In the resulting mixture a quantity of CH_2Cl_2 and H_2O was added and the organic layer was separated, washed with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **2a** (175.7 mg, 68.7%) as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.89–7.02 (3H, m), 5.38 (1H, t, *J*=7.1Hz), 5.20 (1H, t, *J*=7.1Hz), 5.09 (6H, m), 4.57 (2H, s), 3.20 (2H, d, *J*=7.2Hz), 2.27 (3H, s), 2.25 (3H, s), 2.04 (3H, s), 1.98–2.20 (28H, m), 1.65 (3H, s), 1.58 (21H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 171.1 (s), 169.3 (s), 169.2 (s), 148.2 (s), 146.2 (s), 137.6 (s), 135.4 (s), 135.2 (s), 134.9 (s, x2), 134.8 (s), 134.2 (s), 133.4 (s), 131.2 (s), 130.7 (d, x2), 124.8 (d), 124.3 (d), 124.2 (d), 123.9 (d), 123.7 (d), 122.8 (d), 122.6 (d), 120.6 (d), 119.8 (d), 62.0 (t), 39.7 (t), 35.2 (t), 28.5 (t), 26.7 (t), 26.6 (t), 26.5 (t), 26.3 (t), 25.6 (q), 21.1 (q), 20.9 (q), 20.8 (q), 17.6 (q), 16.2 (q), 15.9 (q); IR (film): ν_{max} = 2920, 1770, 1740, 1500, 1450, 1370, 1200 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 275 (850), 203 (6800) nm; EI-MS: *m/z* (% rel. int.) = 796 ([M]$^+$, 2), 736 (4), 465 (16), 397 (24), 189 (42), 161 (78), 135 (95), 69 (100); *Anal.* Calcd for $C_{52}H_{76}O_6$: C, 78.34; H, 9.61. Found: C, 78.30; H, 9.55.

2-[24-acetoxy]-octaisopentyl-1,4-diacetoxy-benzene (**2b**): A solution of **2a** (98.0 mg, 0.12 mmol) in EtOH (10 ml) was hydrogenated using 10% Pd/C under an atmosphere of H_2. The mixture was stirred at 50° C overnight. Then the catalyst was removed by filtration and the solvent was evaporated to afford pure **2b** (89.3 mg, 91.6%)

as a colourless oil; ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.92–6.97 (3H, m), 2.49 (2H, m), 2.29 (3H, s), 2.26 (3H, s), 2.14 (3H, s), 1.10–1.57 (52H, m), 0.85 (3H, d, *J*=6.5Hz), 0.84 (18H, brs), 0.83 (3H, d, *J*= 6.5Hz); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 169.3 (s, x3), 148.2 (s), 146.2 (s), 136.2 (s), 123.0 (d), 122.8 (d), 119.7 (d), 39.3 (t), 37.5 (t), 37.4 (t), 37.2 (t), 32.8 (d), 27.9 (d), 27.8 (t), 24.8 (t), 24.5 (t), 22.7 (q), 22.6 (q), 21.1 (q), 20.8 (q), 19.8 (q), 19.7 (q), 19.6 (q), 19.5 (q); IR (film): ν_{max} = 2920, 1770, 1740, 1500, 1470, 1370, 1220 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 266 (1016) nm; EI-MS: *m/z* (% rel. int.) = 812 ([M]$^+$, 1), 755 (1), 712 (10), 670 (42), 122 (73), 71 (78), 57 (100); *Anal.* Calcd for $C_{52}H_{92}O_6$: C, 76.78; H, 11.41. Found: C, 76.81; H, 11.44.

2-[24-oxy]-octaprenyl-1,4-quinone (**2c**) & *2-[24-hydroxy]-octaprenyl-1,4-quinone* (**2d**): A quantity of **2** (205.0 mg, 0.30 mmol) in acetone (3 ml) was added to a solution of CrO_3 (100 mg, 1.0 mmol) in 70% HOAc (10 ml). The resulting mixture was stirred at 50° C overnight before a volume of CH_2Cl_2 and H_2O was added. The organic layer was separated, washed with saturated NH_4Cl and NaCl solutions, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (cyclohexane/EtOAc) to afford **2c** (138.5 mg, 69.3%) and **2d** (47.4 mg, 23.6%) as colourless oils; (**2c**): ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 10.06 (1H, s), 6.71 (1H, d, *J*=2.2Hz), 6.51–6.47 (2H, m), 5.08 (8H, m), 3.10 (2H, d, *J*= 7.1 Hz), 2.62 (2H, m), 1.97–2.11 (26H, m), 1.65 (3H, s), 1.60 (3H, s), 1.57 (18H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 190.9 (d), 187.8 (s), 187.5 (s), 149.1 (s), 140.1 (t), 139.7 (s), 136.7 (s), 136.3 (s), 135.4 (s), 134.7 (s), 133.0 (s), 132.3 (d), 131.2 (s), 125.9 (d), 124.3 (d), 124.1 (d), 123.7 (d), 123.4 (d), 117.6 (d), 39.7 (t), 39.6 (t), 39.5 (t), 39.4 (t), 30.3 (t), 29.6 (t), 27.3 (t), 27.1 (t), 26.7 (t), 26.6 (t), 26.4 (t), 25.6 (t), 25.2 (q), 17.6 (q), 16.0 (q), 15.9 (q); IR (film): ν_{max} = 2920, 1720, 1660, 1440, 1380, 1200 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 233 (6666), 205 (15.583) nm; EI-MS: *m/z* (% rel. int.) = 666 ([M]$^+$, 3), 600 (8), 225 (4), 217 (8), 123 (19), 84 (31), 57 (42), 43 (100); *Anal.* Calcd for $C_{46}H_{66}O_3$: C, 82.82; H, 9.98. Found: C, 82.91; H, 10.04. (**2d**): ^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 6.68–6.76 (2H, m), 6.50 (1H, d, *J*=1.8Hz), 5.27 (1H, t, *J*=6.5Hz), 5.07 (7H, m), 4.07 (2H, brs), 3.09 (2H, d, *J*=6.5Hz), 1.96–2.15 (28H, m), 1.64 (3H, s), 1.59 (3H, s), 1.56 (18H, brs); ^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 187.9 (s), 187.6 (s), 148.5 (s), 140.2 (s), 138.3 (s), 136.7 (s), 136.3 (s), 135.5 (s), 135.4 (s), 135.0 (s), 134.9 (s), 134.4 (s), 132.3 (d), 131.2 (s), 128.6 (d), 124.8 (d), 124.3 (d), 124.2 (d), 124.1 (d), 123.9 (d), 123.7 (d), 117.5 (d), 60.3 (t), 39.9 (t), 39.7 (t), 35.2 (t), 27.3 (t), 27.0 (t), 26.7 (t), 26.6 (t), 26.4 (t), 26.2 (t), 25.7 (q), 17.7 (q), 16.2 (q), 16.1 (q), 16.0 (q); IR (film): ν_{max} = 3300, 2916, 1665, 1443, 1378, 1296 cm^{-1}; UV(C_6H_{14}): λ_{max} (ε) = 245 (21810), 211 (33783) nm; EI-MS: *m/z* (% rel. int.) = 668 ([M]$^+$, 9), 650 (6), 514 (3), 310 (6), 201 (11), 160 (100), 122 (15), 69 (22); *Anal.* Calcd for $C_{46}H_{68}O_3$: C, 82.57; H, 10.25. Found: C, 82.56; H, 10.22.

Cytotoxicity assays

In vitro cytotoxicity experiments were performed on the cell line (human non-small-cell lung cancer) L16 (Roussakis *et al.*, 1991) and its clone C98 (Siavoshian *et al.*, 1998). The C98 clone represents phenotypes and genotypes similar to that of the cell line L16 but differs from that line in regards to cell doubling time. Both of them were performed according to NCI procedures (Geran *et al.*, 1972) using the colorimetric assay based on conversion of tetrazolium dye (MTT) to a blue formazan product using live mitochondria (Mosmann, 1983).

Antibacterial activity

The bacteriostatic activities were determined by the disc diffusion method (Chinou *et al.*, 1994) against two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853). Standard antibiotics Ciprof loxacine (CIP), Imipenem (IPM), Amoxicilline with clavulanic acid (AMC) and Netilmicine (Sanofi, Diagnostics Pasteur) were used in order to control the sensitivity of the test organisms. Technical data have been described previously (Cruickshank *et al.*, 1975).

Results and Discussion

The lack of extensive biological data on polyprenyl-hydroquinones prompted us to undertake a

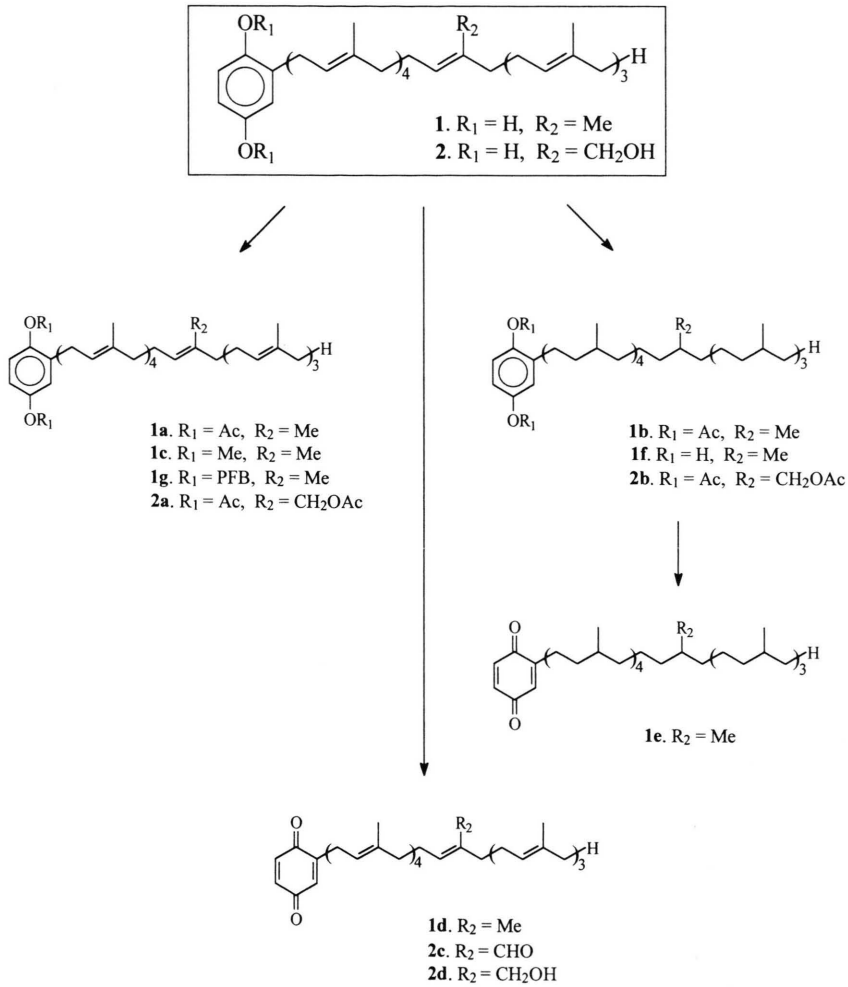

Fig. 1. Structural modifications of natural products **1** and **2**.

comprehensive study on the structure activity relationships between the natural products **1** and **2**, isolated from the sponge *Ircinia spinosula* and the above described eleven derivatives. All of them were tested against cancer cell line L16 and its clone C98 for cytotoxicity. From the two natural products only metabolite **2** showed a moderate activity against the clone C98 (compound **1**: inactive, compound **2**: 9.1 μ g/ml). Among all synthesized derivatives **2d** was proved to be the most interesting one (17.4 μ g/ml against C98 clone), while cancer line L16 was appeared to be resistant to all tested compounds. As a general comment, can be stated the necessity of free hydroxyl groups either on the aromatic ring or the side chain of the compounds, in order to exhibit considerable cytotoxicity.

All thirteen natural and synthetic polyprenylhydroquinones were also tested against six standard bacterial strains (Table I). The antibacterial studies showed that even though **1** was completely inactive, its derivatives exhibit a wide range of activity. The oxidation of the hydroquinone moiety to quinone increased the activity against *E. coli*. The hydrogenation of the double bonds on the side chain of analogues **1a** and **2a** increased in the activity of the resulting derivatives **1b** and **2b**. Metabolite **2** showed a specific activity against *S. aureus* and *E. cloacae*. The activity trend for the derivatives of compound **2** was found to be similar

Table I. Bacteriostatic activity of the polyprenylated marine hydroquinones and their derivatives.

Compounds	*S.aureus*	*S. epidermidis*	*P.aeruginosa*	*E.cloacae*	*K.pneumoniae*	*E.coli*
1	–	–	–	–	–	–
1a	–	–	–	–	–	–
1b	24	24	28	28	–	–
1c	28	–	14	12	–	–
1d	26	30	30	–	–	32
1e	18	12	22	30	–	22
1f	–	–	–	–	–	–
1g	–	–	32	30	–	–
2	30	–	–	16	–	–
2a	–	–	–	–	–	–
2b	20	22	26	20	–	–
2c	18	20	18	20	–	–
2d	20	26	26	28	–	–
CIP[1]	31	28	28	31	28	28
IPM[2]	30	28	31	29	26	26
AMC[3]	32	30	31	31	25	29

[1] Ciprofloxacine (CIP)
[2] Imipenem (IPM)
[3] Amoxicilline with clavulanic acid (AMC).

to that of metabolite **1**. These results are in accordance with the literature reports (De Rosa *et al.*, 1994) for polyprenylhydroquinones confirming the moderate to strong antimicrobial effects and clearly showing that both the oxidation of the hydroquinone to quinone and the hydrogenation of the side-chain double bonds increases the pharmacological potency of the molecules.

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