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SULFATED POLYPRENYLHYDROQUINONES FROM THE SPONGE *IRCINIA SPINOSULA*

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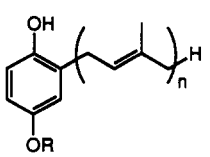
ABSTRACT.—Three sodium polyprenylhydroquinone sulfates with related previously described polyprenylhydroquinones have been isolated from the sponge *Ircinia spinosula*, collected in the Adriatic Sea. The structures were proposed by means of spectroscopic data and by chemical correlation. Brine shrimp and fish lethalities of isolated compounds are reported.

Marine organisms have provided a large number of compounds of mixed biogenesis, originating partly from mevalonate and partly from a benzenoid precursor. A number of linear or cyclic prenylhydroquinones have been described with a terpenoid portion, ranging from one to eight isoprene units. Linear polyprenylhydroquinones (from four to eight isoprene units) have been isolated from *Ircinia* spp. sponges (1–4). Recently, 2-polyprenylhydroquinone sulfates were reported from *Dysidea* sp. (5) and *Sarcotragus spinulosus* sponges (6).

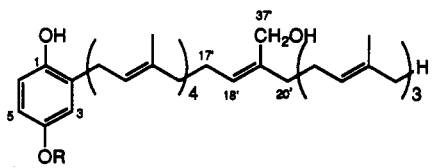
In the course of our search for marine natural compounds having biological activity we have investigated the marine sponge *Ircinia spinosula* Schulze (order Dictyoceratida, family Spongiidae) collected near Zadar, Croatia, whose extract showed activity (LD_{50}=19 μg/ml) in a brine shrimp assay (7,8). By fractionating the extract, we isolated three 2-polyprenylhydroquinones [**1–3**], the hydroxylated 2-octaprenylhydroquinone **4**, and three 2-polyprenylhydroquinone sodium sulfates [**5–7**]. From the same sponge, collected in the Tyrrhenian Sea, other authors have reported the isolation and structural determination of **1–3**, in the form of a mixture, and of **4** (2). Recently we have reported the separation by hplc, and some biological activities of **1–3** (9); we describe herein the isolation and structural elucidation of the new compound **7**, and certain biological activities of the compounds obtained.

The Et_2O-soluble fraction of the Me_2CO extract of *I. spinosula* was chromatographed on Si gel to give three phenolic fractions, each of which gave a single spot on tlc. In order of polarity these were a mixture of 2-prenylhydroquinones [**1–3**], the hydroxylated 2-octaprenylhydroquinone **4**, and a mixture of sulfated 2-prenylhydroquinones [**5–7**]. The mixtures were separated by hplc; the less polar fraction yielded the previously described compounds **1–3** (9), while the more polar fraction, after repeated hplc, yielded compounds **5–7**.

A preliminary nmr spectral analysis of all isolated compounds showed them to have a close similarity, and strongly supported the presence of a 2-polyprenylhydroquinone skeleton in each.



1 R=H n=6
2 R=H n=7
3 R=H n=8
5 R=SO_3Na n=7
6 R=SO_3Na n=8



4 R=H
7 R=SO_3Na

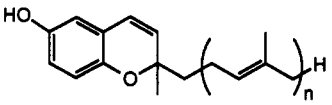
Compounds **1–4** showed the same uv (294 nm) and ir (3350, 1500, 1445, 910, 785, 730 cm^{-1}) spectra, indicating the presence of a monosubstituted hydroquinone structure. This was supported by the occurrence of three aromatic protons in the ^{1}H-nmr spectrum, namely, two doublets at δ 6.67 (J=8.5 Hz) and 6.61 (J=2.8 Hz), and a double doublet at δ 6.57 (J=8.5 and 2.8 Hz). All spectral data of **1–4** were in agreement with published values (2).

Compounds **5–7** were obtained as amorphous solids, and showed the same uv (284 nm) and ir (3350, 1500, 1240, 840 cm^{-1}) spectra, indicating the presence of a substituted sulfated hydroquinone moiety. This was supported by the observation in their ^{1}H-nmr spectra of two doublets at δ 7.01 (J=2.8 Hz) and 6.63 (J=8.5 Hz) and a double doublet at δ 6.98 (J=8.5 and 2.8 Hz), and in their ^{13}C-nmr spectra of six aromatic carbons, three doublets (δ 123.5, 120.4, 116.2) and three singlets (δ 152.5, 144.4, 128.8). The ^{13}C-nmr chemical shifts of the aromatic carbons were in agreement with those reported for the 4-monoacetyl derivative of 2-prenylhydroquinone (10), suggesting that these compounds had the 4-hydroxy phenol group esterified. Their high polarity suggested that the phenolic hydroxy group was esterified with an inorganic acid. Their negative-ion fabms, exhibiting quasi-molecular anion peaks $[M-H]^-$ at *m/z* 687, 755, and 771, and anion peaks $[M-Na]^-$ at *m/z* 665, 733, and 749, for **5–7**, respectively, indicated a sodium salt of heptaprenylhydroquinone [**5**], octaprenylhydroquinone [**6**], and hydroxylated octaprenylhydroquinone sulfate esters [**7**], respectively.

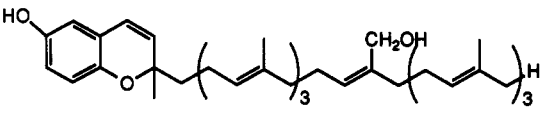
The ^{1}H- and ^{13}C-nmr spectra of **5** and **6** showed the presence of an all-trans-polyprenylic side-chain linked to a hydroquinone moiety, and are in agreement with the data reported for 2-heptaprenylhydroquinone and 2-octaprenylhydroquinone 4-sodium sulfates recently isolated from the deep-water sponge *S. spinulosus* (6).

The ^{1}H- and ^{13}C-nmr spectra of compound **7** were similar to those of compounds **5** and **6**, except for the presence of signals at δ 4.09 and δ 60.1, respectively, in the ^{1}H- and ^{13}C-nmr spectra, assigned to the primary alcohol group in the side-chain, and small differences in the chemical shifts around the OH group. The configurations of double bonds were all assigned as *E* by ^{1}H- and ^{13}C-nmr chemical shifts (δ 1.63–1.54 and 17.7–16.0 for ^{1}H and ^{13}C, respectively) of the vinyl methyls. HETCOR experiments allowed us to assign the chemical shifts in the ^{13}C-nmr spectrum.

Solvolysis of compounds **5–7** with pyridine-dioxane (1:1) gave, for each, two compounds, a chromenol and a prenylhydroquinone. The prenylhydroquinones obtained by solvolysis of sulfates **5–7** were spectroscopically identical to the natural compounds **2–4**. The chromenols obtained, **8–10**, showed the same uv (263, 332 nm) and ir (3360, 1620, 1585, 1220 cm^{-1}) spectra, indicating the presence of a [2H]-1-benzopyran (chromene) skeleton, which was supported by the most abundant fragment ion at *m/z* 161 in the mass spectra of compounds **8–10**. The ^{1}H-nmr spectra of compounds **8–10**, with the exception of signals due to the side-chain, were very similar. They showed, excluding the signals due to the prenyl chain, five protons, four doublets at δ 6.64 (J=8.5 Hz), 6.47 (J=2.8 Hz), 6.27 (J=9.8 Hz), and 5.59 (J=9.8 Hz),



8 n=6
9 n=7



10

and a double doublet at δ 6.57 (J=8.5 and 2.8 Hz), a methylene as a multiplet at δ 1.66, and a methyl singlet at δ 1.37. The above ^{1}H- and ^{13}C-nmr data (see Experimental) were in agreement with those of a related known compound (11). The eims of **8** and **9** showed molecular ions at *m/z* 584 and 652, respectively, and the characteristic fragmentation of prenylated hydroquinones, suggesting that compounds **8** and **9** were the chromenols obtained from 2-heptaprenylhydroquinone [**2**] and 2-octaprenylhydroquinone [**3**], respectively, through an oxidative step during solvolysis followed by ring closure.

The eims of compound **10** showed a molecular ion at *m/z* 668 and the characteristic fragmentation of a prenylated hydroquinone, suggesting that **10** was the chromenol of hydroxylated octaprenylhydroquinone, while the presence of fragments at *m/z* 463 (loss of three isoprene units) and 379 (loss of the hydroxylated isoprene unit from the former ion) allowed us to locate the OH group.

The structures of chromenols **8–10** were confirmed by chemical conversion. 2-Heptaprenylhydroquinone [**2**], 2-octaprenylhydroquinone [**3**], and hydroxylated 2-octaprenylhydroquinone [**4**] were treated with pyridine-dioxane (1:1) overnight, to give chromenols **8–10**, which were spectroscopically identical with those obtained by solvolysis of compounds **5–7**.

None of the tested compounds **4–10** showed inhibitory effects against the test microorganisms used in the antimicrobial assays. These results are in agreement with data previously reported for related compounds (9).

The toxicity of compounds **4–10** was tested in the *Artemia salina* shrimp lethality (7,8) and fish lethality (9) assays. The sulfated derivatives [**5–7**] showed greater activity in both bioassays (brine shrimp: LC_{50} 0.02, 0.04, 0.05 ppm; fish: LC_{50} 15.6, 16.9, 19.6 ppm) than the corresponding hydroquinones **2–4** (brine shrimp: LC_{50} 0.91, 0.98, 0.35 ppm; fish: LC_{50} >100 ppm for all three compounds). The greater toxicity of the sulfated derivatives [**5–7**] would suggest that these metabolites play a defensive role against the normal macro-symbionts (e.g., worms, crabs) living in sponges, which in fact are absent in *I. spinulosa*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Varian DMS 90 spectrophotometer. Ir spectra were recorded on a Bio-Rad FTS-7 Ft-ir spectrometer. Eims were recorded on a Fisons Trio 2000 spectrometer, coupled with an Intel computer; fabms were recorded on a VG analytical ZAB2SE double-focusing mass spectrometer, equipped with a cesium gun operating at 25 KeV (2 μA) using glycerol as matrix. ^{1}H- and ^{13}C-nmr spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. The 2D nmr spectra were obtained using Bruker's microprograms. Chromatography was performed using pre-coated Merck F_{254} plates, with Kieselgel 60 powder. Prep. hplc purifications were carried out on a Waters apparatus equipped with a Spherisorb S5 ODS2 column (10 mm×25 cm) and with a refractive index detector. Microorganisms were obtained from DSM, Germany.

ANIMAL MATERIAL.—*Ircinia spinosula* was collected by hand at about 3 m depth at Sutomiscica, Zadar, Croatia, in August 1994, and frozen at −20° until extracted. A voucher specimen is maintained in the institute collection (voucher No. S1ZD).

EXTRACTION AND ISOLATION.—The frozen sponge (100 g dry wt after extraction) was extracted with Me_2CO and, after elimination of the solvent *in vacuo*, the aqueous residue was extracted with Et_2O and then with *n*-BuOH. The Et_2O extract was evaporated *in vacuo* to obtain a brown oil (4.9 g), which was applied to a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether (40–70°) to Et_2O and then with $CHCl_3$-MeOH (9:1).

Fractions with the same tlc profile were combined. Three fractions, positive to reagents for phenolic compounds, were recovered. The less polar fraction was subjected to prep. hplc, as described previously (9), yielding **1** (57 mg), **2** (300 mg), and **3** (170 mg), while **4** (210 mg) was recovered in the second fraction. The third fraction was subjected to prep. hplc (CH_3CN-H_2O, 9:1), yielding subfraction **A** (250 mg) and **7** (660 mg). Further hplc (65% aqueous Me_2CO) of subfraction **A** gave **5** (180 mg) and **6** (60 mg). The identifica-

tion of compounds **1–6** was accomplished by comparison of their spectral data (uv, ir, ms, nmr) with those reported previously (2,6).

Compound **7**.—Amorphous; uv λ max (MeOH) 284 (ε 2900) nm; ir ν max ($CHCl_3$) 3350, 1500, 1240, 840 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.02 (1H, d, J=2.8 Hz, H-3), 6.98 (1H, dd, J=8.5 and 2.8 Hz, H-5), 6.64 (1H, d, J=8.5 Hz, H-6), 5.28 (1H, t, J=7.0 Hz, H-18′), 5.21 (1H, br t, J=4.4 Hz, H-2′), 5.09 (6H, m), 4.09 (2H, br s, H-37′), 3.18 (2H, d, J=4.4 Hz, H-1′), 2.12 (4H, m), 2.02 (12H, m), 1.95 (12H, m), 1.67 (3H, s, H-32′), 1.63 (3H, s, H-33′), 1.58 (6H, s, H-36′, H-38′), 1.56 (9H, s, H-34′, H-35′, H-39′), 1.54 (3H, s, H-40′); ^{13}C nmr ($CDCl_3$) δ 152.5 (s, C-1), 144.4 (s, C-4), 138.0 (s, C-3′), 137.9 (s, C-19′), 135.4 (s), 135.2 (s), 134.9 (s), 134.8 (s), 134.4 (s), 131.2 (s, C-31′), 128.9 (s, C-2), 128.7 (d, C-18′), 124.8 (d), 124.4 (d), 124.2 (d), 124.0 (d), 123.4 (d, C-3), 121.4 (d, C-2′), 120.4 (d, C-5), 116.2 (d, C-6), 60.1 (t, C-37′), 39.8 (t), 35.0 (t, C-20′), 29.5 (t, C-1′), 26.8 (t), 26.5 (t), 26.2 (t), 25.7 (q, C-32′), 17.7 (q, C-40′), 16.1 (q), 16.0 (q); negative-ion fabms m/z $[M-H]^-$ 771 (12), $[M-Na]^-$ 749 (100); positive-ion fabms $[M+Na]^+$ 795 (100), $[M+H]^+$ 773 (30), $[M-SO_3Na+Na]^+$ 693 (23), 675 (15).

SOLVOLYSIS OF COMPOUNDS **5–7**.—Compounds **5–7** (50 mg each) were heated in a mixture of dioxane (5 ml) with pyridine (5 ml) at 110° for 1 h. The cooled solutions were neutralized with 2 N HCl and extracted with $CHCl_3$ (3×5 ml). The combined extracts were evaporated *in vacuo* and the residues were chromatographed on a Si gel column, eluted with petroleum ether-Et_2O (4:1) to give for each mixture a chromenol [**8–10**] (20 mg each), and a prenylhydroquinone [**2–4**] (25 mg each). The prenylhydroquinones were spectroscopically (uv, ir, ms, nmr) identical to the natural compounds **2–4**.

Chromenol **8**.—Uv λ max (MeOH) (ε) 263 (3000), 332 (2200) nm; ir ν max ($CHCl_3$) 3360, 1620, 1585, 1220 cm^{-1}; eims m/z $[M]^+$ 584 (18), $[M-Me]^+$ 567 (20), 515 (5), 447 (3), 379 (3), 311 (16), 243 (25), 175 (33), 161 (100); ^1H nmr ($CDCl_3$) δ 6.64 (1H, d, J=8.5 Hz, H-8), 6.57 (1H, dd, J=8.5 and 2.8 Hz, H-7), 6.47 (1H, d, J=2.8 Hz, H-5), 6.27 (1H, d, J=9.8 Hz, H-4), 5.59 (1H, d, J=9.8 Hz, H-3), 5.10 (6H, m), 2.06 (12H, m), 1.98 (12H, m), 1.68 (3H, s), 1.66 (2H, overlapped m), 1.60 (9H, s), 1.59 (3H, s), 1.58 (3H, s), 1.56 (3H, s), 1.37 (3H, s, H_3-11); ^{13}C nmr ($CDCl_3$) δ 149.1 (s), 147.0 (s), 135.1 (s), 134.9 (s), 131.0 (d), 130.8 (s), 124.3 (d), 124.2 (d), 122.6 (d), 116.7 (d), 115.4 (d), 112.8 (d), 40.9 (t), 39.7 (t), 29.7 (t), 26.7 (t), 26.0 (q), 25.7 (q), 22.6 (t), 17.7 (q), 16.0 (q).

Chromenol **9**.—Uv λ max (MeOH) (ε) 262 (3100), 330 (2100) nm; ir ν max ($CHCl_3$) 3360, 1620, 1585, 1220 cm^{-1}; eims m/z $[M]^+$ 652 (13), $[M-Me]^+$ 637 (15), 583 (5), 515 (3), 447 (3), 379 (3), 243 (19), 175 (30), 161 (100); ^1H- and ^{13}C-nmr ($CDCl_3$) data were similar to those of chromenol **8**, except for the intensity of signals assigned to vinyl methylenes and methyls, due to the presence of an additional isoprene unit.

Chromenol **10**.—Uv λ max (MeOH) (ε) 264 (3200), 334 (2300) nm; ir ν max ($CHCl_3$) 3360, 1620, 1585, 1220 cm^{-1}; eims m/z $[M]^+$ 668 (8), $[M-Me]^+$ 653 (8), $[M-H_2O]^+$ 650 (15), 583 (4), 515 (3), 463 (4), 379 (5), 311 (10), 243 (12), 175 (29), 161 (100); ^1H nmr ($CDCl_3$) δ 6.64 (1H, d, J=8.5 Hz), 6.57 (1H, dd, J=8.5 and 2.8 Hz), 6.47 (1H, d, J=2.8 Hz), 6.27 (1H, d, J=9.8 Hz), 5.59 (1H, d, J=9.8 Hz), 5.10 (6H, m), 2.06 (12H, m), 1.98 (12H, m), 1.68 (3H, s), 1.66 (2H, overlapped m), 1.60 (9H, s), 1.59 (3H, s), 1.58 (3H, s), 1.56 (3H, s), 1.37 (3H, s); ^{13}C nmr ($CDCl_3$) δ 149.1 (s), 147.0 (s), 135.1 (s), 134.9 (s), 131.0 (d), 130.8 (s), 124.3 (d), 124.2 (d), 122.6 (d), 116.7 (d), 115.4 (d), 112.8 (d), 40.9 (t), 39.7 (t), 29.7 (t), 26.7 (t), 26.0 (q), 25.7 (q), 22.6 (t), 17.7 (q), 16.0 (q).

SOLVOLYSIS OF COMPOUNDS **2–4**.—Compounds **2–4** (20 mg each) were heated in a mixture of dioxane (5 ml) and pyridine (5 ml) at 110° overnight. The cooled solutions were neutralized with 2 N HCl and extracted with $CHCl_3$ (3×5 ml). The combined extracts were evaporated *in vacuo* to obtain, separately, chromenols **8–10** (15 mg each), which were spectroscopically (uv, ir, ms, nmr) identical to the chromenols obtained by solvolysis of sulfates **5–7**.

BIOLOGICAL EVALUATION.—*Antimicrobial and antifungal activities*.—Three Gram-positive bacteria [*Bacillus subtilis* (DSM 347), *Micrococcus luteus* DSM 348), and *Pseudomonas aeruginosa* (DSM 1117)], a Gram-negative bacterium [*Escherichia coli* (DSM 1103)], and a fungus [*Candida albicans* (DSM 1665)] were used for the antimicrobial assays, as described previously (9). None of the tested compounds [**4–10**] showed activity in preliminary diffusion tests with 100 μg of compound per paper disk.

Brine shrimp lethality.—A brine shrimp (*Artemia salina*) lethality assay performed as already described (7,8), gave the following data: **2** (LC_{50}=0.91 ppm), **3** (LC_{50}=0.98 ppm), **4** (LC_{50}=0.35 ppm), **5** (LC_{50}=0.02 ppm), **6** (LC_{50}=0.04 ppm), **7** (LC_{50}=0.05 ppm), **8** (LC_{50}=1.15 ppm), **9** (LC_{50}=1.45 ppm), and **10** (LC_{50}=32.3 ppm).

Fish lethality.—The fish (*Gambusia affinis*) lethality assay performed as described previously (9), gave the following data: **5** (LC_{50}=15.6 ppm), **6** (LC_{50}=16.9 ppm), **7** (LC_{50}=19.6 ppm), and **2–4**, **8–10** (LC_{50}=>100 ppm).

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