A New Tetraprenylhydroquinone Derivative with an Acetic Acid Unit from the Marine Sponge *Ircinia muscarum*

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A new linear tetraprenylhydroquinone derivative (**3**) with an acetic acid unit has been isolated from the marine sponge *Ircinia muscarum*. The structure of **3** was determined by spectral analysis.

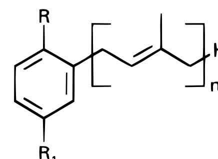
Linear prenylated hydroquinones and related secondary metabolites have been isolated previously from sponges,[1−7] tunicates,[8−10] and algae.[11−12] Earlier reports on the testing of prenylhydroquinones such as geranylhydroquinone ($n = 2$) had shown that in test animals the preadministration of such compounds prevented the induction of some forms of leukemia, Rous sarcoma, and mammary carcinoma.[8,13] In this paper we report the isolation and structural elucidation of a new hydroquinone derivative (**3**) from the Mediterranean sponge *Ircinia muscarum* Vacelet, 1959 (Irciniidae).

An Me_2CO extract of the frozen sponge was partitioned between Et_2O and H_2O. From the Et_2O extract, the known compounds **1** and **2** were isolated and identified by comparison of their spectral data with literature values.[2] In addition, a minor component was isolated and further identified as the new metabolite **3**.

Compound **3** was obtained as a colorless oil and had a molecular formula of $C_{28}H_{40}O_3$, established by positive ion FABMS at m/z 425 $[M + H]^+$. The ^1H- and ^{13}C-NMR spectra contained signals characteristic for a 2-tetraprenyl-1,4-hydroquinone derivative, incorporating an all-trans geometry.[14] This was confirmed by the UV spectrum (λ max 279 nm, ϵ 1064). In addition, a 2H singlet signal was seen at 3.55 ppm in the ^1H-NMR spectrum. In the ^{13}C-NMR spectrum, signals were seen at 40.17 ppm and 177.94 ppm corresponding with an isolated CH_2 group and a carboxylic acid group, respectively. The presence of an acid was further corroborated by the IR spectrum (υ max 3600−2500 br, 1690 cm^{-1}). The placement of this CH_2COOH group at C-4 was achieved by a long-range HETCOR experiment. Correlations were observed between the proton signal at 3.55 ppm (H-7) and the carbonyl carbon signal at 177.94 ppm (C-8) as well as the ring carbon signals at 125.27 (C-4), 128.38 (C-3), and 130.92 (C-5) ppm.

Compound **3** is an inhibitor of the enzyme system TOPO II[15] ($IC_{50} = 0.5$ μg/mL) but did not show any significant in vitro antitumor activity in the cell lines studied:[16] P-388 (murine leukemia); A-549 (human lung carcinoma); HT-29 (human colon carcinoma); and MEL-28 (human melanoma).

This is the first report of a hydroquinone substituted with an alkyl carboxylic acid. Because **3** occurs with the other known hydroquinone derivatives **1**, **2**, and **4** (Figure 1), it is reasonable to hypothesize that all are biosynthesized along the same metabolic pathway, starting from *p*-hydroxybenzoic acid as the ring precursor.[17] It is also possible, however, that **3** may be derived from the transformation (deamination, decarboxylation, and oxidation) and prenylation of tyrosine.



1: R = R$_1$ = OH, n = 4
2: R = OH, R$_1$ = COOH, n = 4
3: R = OH, R$_1$ = CH_2COOH, n = 4
4: R = OSO_3Na, R$_1$ = OH, n = 6

Figure 1. Structures of linear tetraprenylhydroquinones **1**−**2** and derivatives **3**−**4**.

Experimental Section

General Experimental Procedures. The ^1H-NMR spectra were run at 299.95 MHz and ^{13}C-NMR spectra at 75.43 MHz in $CDCl_3$ unless otherwise stated, using a Varian Unity 300 spectrometer. Chemical shifts (δ, ppm) are referenced to solvent peaks: δ_H 7.26 and δ_C 77.0 (residual $CDCl_3$). DEPT and $^1H-^{13}C$ HETCOR NMR experiments[18,19] were performed on this same NMR spectrometer, using standard Varian pulse sequences. MS were determined with VG ZAB-SE (LRFAB), 70-VSE (HRFAB), and 70-SE-4F (MS/MSFAB) spectrometers. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 15 double beam spectrometer. Si gel 60 (70−230 mesh, Merck) and Prepex 40−63 C-18 were used for column chromatography. Si gel 60 F_{254} and RP-18 $F_{254}S$ (Merck) plates were used for TLC. Vanillin reagent and UV light (254 nm) were used for spot detection. HPLC was carried out using a Waters 991 photodiode-array detector and a Rheodyne injector.

Sponge Collection. The sponge (*Ircinia muscarum*) was collected by scuba at Columbretes, Baleares, Spain, and immediately frozen. A voucher specimen (08/10/88 1−007) is on file at Pharma Mar S. A.

Extraction And Isolation. The frozen sponge (500 g, wet wt) was extracted exhaustively by homogenization with Me_2CO in a blender at room temperature. The extract was filtered and then concentrated. The oily

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aqueous residue was partitioned against Et_2O. The Et_2O-soluble fraction was concentrated *in vacuo* to give a brown oil (8.5 g) that was subjected to normal-phase vacuum–liquid chromatography (stepwise gradient elution from hexane to CH_2Cl_2 to EtOAc to MeOH).

A major fraction (5.6 g) eluting with CH_2Cl_2 showed in vitro cytotoxicity against the P-388 cell line. It was rechromatographed on Si gel ($CHCl_3$/MeOH gradient) and further purified by reversed-phase flash chromatography (MeOH/H_2O 9:1) to provide the known compounds **1** (2 g) and **2** (1 g), which were responsible for the activity shown by the crude extract.

A minor fraction (830 mg) eluting with CH_2Cl_2/EtOAc 1:1 from the initial chromatography was further purified on Si gel using a $CHCl_3$/MeOH gradient, followed by reversed-phase flash column chromatography (MeOH/H_2O 9:1) to afford **3** (4 mg).

All products were subjected to an analytical HPLC system consisting of a C18 reversed-phase radial pack cartridge (10 μ) eluted with a 95:5 mixture of MeOH/H_2O at 1.5 mL/min: compound **1** (t_R 3.30 min, λ 294 nm), compound **2** (t_R 3.60 min, λ 258 nm), and compound **3** (t_R 3.66 min, λ 279 nm).

1,4-Dihydroxy-2-tetraprenylbenzene derivative 3: HRFABMS *m/z* 425.3055 (calcd for $C_{28}H_{40}O_3$ *m/z* 425.3039); IR (υ max, KBr) 3600–2500 br, 3420, 1690, 1610, 1500, 1440, 1265 cm^{-1}; UV (λ max, MeOH 279, ϵ 1064); ^1H NMR ($CDCl_3$) δ 7.02 (1H, dd, $J = 7.5$, 2.1 Hz, H-5), 7.01 (1H, dd, $J = 2.1$, 0.9 Hz, H-3), 6.76 (1H, dd, $J = 7.5$, 0.9 Hz, H-6), 5.32(1H, t, $J = 7.2$ Hz, H-2′), 5.09 (3H, m, H-6′, H-10′, H-14′), 3.55 (2H, s, H-7), 3.35 (2H, d, $J = 7.2$ Hz, H-1′), 2.16–1.94 (12H, m, H-4′, 5′, 8′, 9′, 12′, 13′), 1.77 (3H, s, Me-20′), 1.68 (3H, s, Me-17′), and 1.60 (9H, s, Me-16′, 18′, 19′); ^{13}C NMR ($CDCl_3$) δ 177.94 (C-8), 153.78 (C-1), 138.79 (C-3′), 135.57[a] (C-7′), 134.90[a] (C-11′), 131.26 (C-2), 130.92 (C-5), 128.38 (C-3), 126.98 (C-15′), 125.27 (C-4), 124.36[b] (C-14′), 124.19[b] (C-10′), 123.60[b] (C-6′), 121.35 (C-2′), 116.02 (C-6), 40.17 (C-7), 39.68[c] (C-12′), 39.65[c] (C-4′, 8′), 29.85 (C-1′), 26.72[d] (C-13′), 26.56[d] (C-9′), 26.38[d] (C-5′), 25.68 (C-17′), 17.67 (C-16′), 16.24 (C-20′), 16.03[e] (C-18′) and 15.99[e] (C-19′) (values with the same superscript may be interchanged).

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