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A NEW CYTOTOXIC PRENYLHYDROQUINONE FROM A MEDITERRANEAN TUNICATE OF THE GENUS *APLYDIUM*

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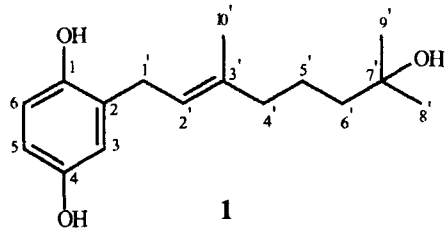
Abstract: The mediterranean tunicate *Aplydium sp.* contains a new hydroxydiprenylhydroquinone **1** together with the known compound 2-[(2'*E*, 6'*E*)-3',7'-dimethylocta-2',6'-dienyl]benzene-1,4-diol (**2**). The structure of **1** was elucidated by interpretation of spectral data. Both compounds showed significant cytotoxicity against four tumor cell lines, in particular, against P-388 mouse lymphoma suspension culture.

Key Words: Prenylhydroquinone, tunicate, *Aplydium*, cytotoxicity.

INTRODUCTION

Tunicates have been considered one of the most interesting groups among the marine invertebrates that produce natural products with promising bioactivities. In particular, tunicates of the genus *Aplydium* have given rise to an array of structurally diverse and biologically active metabolites.[1] *Aplydium* tunicates have been reported to contain prenylhydroquinones[2-4] an otherwise typically sponge derived group of compounds.



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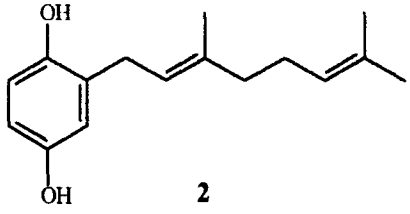

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RESULTS AND DISCUSSION

As a part of our research project focussing on the discovery of new cytotoxic metabolites from marine organisms of the southern coast of Spain we obtained specimens of an unidentified tunicate of the genus *Aplydium*. The chemical study of this organism has led to the isolation of the new hydroxydiprenylhydroquinone 1 together with the known compound 2-[(2'*E*,6'*E*)-3',7'-dimethylocta-2',6'-dienyl]benzene-1, 4-diol (**2**).[2]

Compound **1** was isolated as a colourless oil of molecular formula $C_{16}H_{24}O_3$ as indicated by the high resolution mass measurement. A strong hydroxyl band at 3220 cm^{-1} in the infrared spectrum together with the UV absorption at 294 nm indicated the presence of a hydroquinone nucleus. The presence of a 2-substituted hydroquinone ring system was confirmed by the ^{1}H-NMR signals at δ 6.66 (d, 1H, J = 8.6 Hz), 6.65 (d, 1H, J = 3.1 Hz) and 6.57 (dd, 1H, J = 8.6, 3.1 Hz) and by the ^{13}C signals at δ 149.7 (s), 147.8 (s), 138.2 (s), 121.8 (d), 116.5 (d) and 116.4 (d). The remaining ten carbon atoms were assigned to a diprenyl side chain linked head to tail as follows: the benzylic methylene gave rise to the ^{1}H-NMR signal at δ 3.30 (d, 2H, J = 7.2 Hz) which was coupled with a broad triplet at δ 5.32 (br t, 1H, J = 7.2 Hz) assigned to the olefinic proton of a trisubstituted double bond. The *E* geometry of this double bond was deduced from the chemical shift of the methyl carbon signal at δ 16.2 (q).[5] A singlet at δ 71.4 on the ^{13}C-NMR together with the ^{1}H-NMR signal at δ 1.22 (s, 6H) assigned to two methyl groups attached to carbon bearing oxygen indicated the presence of

an hydroxyl group at the quaternary carbon of the terminal isoprenyl unit. It was therefore proposed that compound **1** was 2-[(2'*E*)-7'-hydroxy-3',7'-dimethyloct-2'-enyl]benzene-1,4-diol.

Prenylhydroquinones, although structurally simple compounds, have demonstrated potential cancer protective properties.[6] We have examined the cytotoxicity of both metabolites **1** and **2** against P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma cell lines. Both compounds showed significant cytotoxicity against the four tumor cell lines mentioned (see Experimental Section) with a certain selectivity against P-388 mouse lymphoma suspension culture. Furthermore, these results indicate that the hydroxylation on the prenyl chain causes a significant loss in cytotoxicity.

EXPERIMENTAL SECTION

Collection, Extraction and Isolation Procedures. Specimens of *Aplydium sp.* were collected by hand using SCUBA in Cabo de Gata (Almería, Spain) and immediately frozen. The frozen tunicate was extracted exhaustively with acetone at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oily residue (220 mg) which was chromatographed on a SiO_2 column using solvents of increasing polarity from hexane to diethyl ether. Fractions eluted with 40% ether in hexane contained **2** (16 mg, 0.386%) which was further purified by normal phase HPLC eluting with hexane/AcOEt (85:15).[7] Fractions eluted with Et_2O were subjected to normal phase HPLC separation eluting with hexane/AcOEt (6:4) to yield **1** (5 mg, 0.120%).

2-[(2'*E*)-7'-hydroxy-3',7'-dimethyloct-2'-enyl]benzene-1,4-diol (1): Colourless oil; UV (MeOH) λ_{max} 294 (ε = 4250) nm; IR (dry film) ν_{max} 3220, 1657, 1607, 1520 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.66 (d, 1H, *J* = 8.6 Hz, H-6), 6.65 (d, 1H, *J* = 3.1 Hz, H-3), 6.57 (dd, 1H, *J* = 8.6, 3.1 Hz, H-5), 5.32 (br t, 1H, *J* = 7.2 Hz, H-2'), 3.30 (d, 2H, *J* = 7.2 Hz, H-1'), 2.07 (m, 2H, H-4'), 1.72 (br s, 3H, H-10'), 1.50 (m, 4H, H-5' and H-6'), 1.22 (6H, s, H-8' and H-9'); ^{13}C-NMR ($CDCl_3$, 100 MHz) (values with the same superscript are interchangeable) δ 149.7[a] (s, C-4), 147.8[a] (s, C-1), 138.2 (s, C-2), 128.1 (s, C-3'), 121.8 (d, C-3), 116.5[b] (s, C-5),

116.4[b] (s, C-6), 113.6 (d, C-2'), 71.4 (s, C-7'), 42.9[c] (t, C-4'), 39.7[c] (t, C-6'), 29.2 (2q, C-8' and C-9'), 29.0 (t, C-1'), 22.4 (t, C-5'), 16.2 (q, C-10'); HREIMS obs *m/z* = 264.1729 [M+], $C_{16}H_{24}O_3$ requires *m/z* = 264.1725.

Cytotoxicity Assays. The new compounds were tested against four tumor cell lines. The individual cell lines identifiers are given along with the corresponding IC_{50} (μg/ml) values for the tested compound. **2-[(2'*E*)-7'-hydroxy-3',7'-dimethyloct-2'-enyl]benzene-1,4-diol (1)**: P-388 (1.2), A-549 (5), HT-29 (2), MEL-28 (2). **2-[(2'E, 6'E)-3', 7'-dimethylocta-2', 6'-dienyl]benzene-1,4-diol (2)**: P-388 (0.2), A-549 (1), HT-29 (1), MEL-28 (1).

ACKOWLEDGEMENTS

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7.- The hydroquinone **2** is readly oxidized to the corresponding quinone. This quinone was isolated from less polar fractions of the general chromatography due to the oxidation of **2** during the isolation process.