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Avinosol, A Meroterpenoid-Nucleoside Conjugate with Antiinvasion Activity Isolated from the Marine Sponge *Dysidea* sp.

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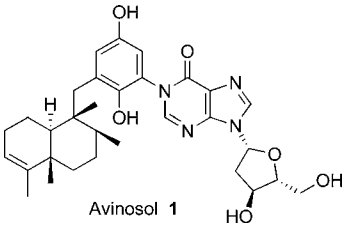
ABSTRACT



Avinosol **1**

The new meroterpenoids avinosol (1), 3′-aminoavarone (2), and 3′-phenethylaminoavarone (3) have been isolated from the marine sponge *Dysidea* sp. collected in Papua New Guinea, and their structures were elucidated by analysis of spectroscopic data. Avinosol (1), which is apparently the first example of a naturally occurring meroterpenoid-nucleoside conjugate, showed antiinvasion activity in a cell-based assay.

Angiogenesis and metastasis are pivotal steps in the lethal progression and spreading of solid tumors.[1] Both processes involve the invasion and migration of either vascular endothelial cells or tumor cells through the extracellular matrix, and as a consequence, inhibition of tissue invasion is an attractive target for developing anticancer drugs. As part of an ongoing program designed to find new antiangiogenic and antimetastatic agents,[2] we have screened a library of crude marine invertebrate extracts in a cell-based assay[3] that detects compounds capable of inhibiting invasion of the extracellular matrix but are not overtly cytotoxic. Crude extracts of the sponge *Dysidea* sp. collected in Papua New Guinea showed promising activity in the assay. Bioassay-guided fractionation

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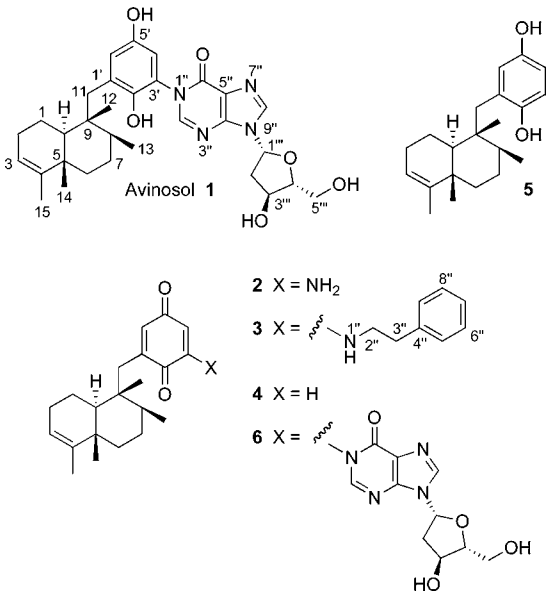
identified the new meroterpenoid-nucleoside conjugate avinosol (**1**) as the antiinvasive compound in the extract. Two additional new meroterpenoids, the selective cytotoxin 3′-aminoavarone (**2**) and the phenethylamine analogue **3**, were also present in the *Dysidea* sp. extract along with the known meroterpenoids avarone (**4**) and avarol (**5**).[4] Details of the isolation and structure elucidation of **1**, **2**, and **3** and the biological activities of **1** and **2** are presented below.

Specimens of *Dysidea* sp. (113 g wet wt) were collected by hand using SCUBA in Papua New Guinea, frozen on site, and transported to Vancouver over dry ice. Thawed sponge specimens were exhaustively extracted with MeOH (3 × 500 mL), and the combined MeOH extracts were concentrated in vacuo to give a dark red residue (4.9 g). The residue was chromatographed on a reversed-phase Amberchrom HP-20 column (step gradient from H_2O to MeOH) to give six fractions. A fraction eluted with 100% MeOH showed antiinvasion activity. Sephadex LH-20 chromatography (eluent MeOH) of the antinvasive material (622 mg) gave a small group of active fractions that were combined (105 mg) and further purified via reversed-phase flash chromatography (Waters Sep-pak, 10 g, step gradient from 3:7 H_2O/CH_3CN to CH_3CN) to give pure avinosol (**1**, 2.0 mg) and a mixture of other compounds. This mixture gave 3′-aminoavarone (**2**, 0.9 mg), the phenethylamine analogue **3**, avarone (**4**), and avarol (**5**) after further reversed-phase HPLC fractionation (CSC-Inertsil 150A/ODS2; 7:3 CH_3CN/H_2O).

Avinosol (**1**) was obtained as a colorless glass that gave a $[M + Na]^+$ ion at *m*/*z* 587.2847 in the HRESIMS consistent with a molecular formula of $C_{31}H_{40}N_4O_6$ (calcd for $C_{31}H_{40}N_4O_6Na$, 587.2846) requiring 14 sites of unsaturation. The ^{13}C NMR spectrum of avinosol (**1**) showed more than the 31 resonances expected from the HRMS analysis, and many of the resonances appeared to be twinned, suggesting the existence of two slowly interconverting forms of the molecule. Similarly, a number of resonances in the ^1H spectrum of **1** (δ 8.37, s, H-8″; 8.36, s, H-8‴; 8.07, s, H-2″; 8.14, s, H-2‴; 6.57, d, J = 2.5 Hz, H-4′; 6.55, d, J = 2.5, Hz, H-4′) integrated for one-half of a proton relative to an upfield methyl singlet (δ 0.87, Me-12) used as an internal standard, and each of these resonances was paired with a second resonance of identical multiplicity that also integrated for one-half a proton.

Analysis of the 1D and 2D NMR data obtained for avinosol (**1**) identified a sesquiterpenoid fragment (C-1 to C-15) identical to that found in the co-occurring metabolites avarone (**4**) and avarol (**5**). HMBC correlations observed from the H-11 (δ 2.70 and 2.77) terpenoid resonances to carbon resonances at δ 132.0 (C-1′), 146.0 (C-2′) and 122.4 (C-6′) demonstrated that the sesquiterpenoid fragment in **1** was linked to an aromatic fragment as in **5** (Figure 1 and Supporting Information). The carbon resonance at δ 122.4 (C-6′) was correlated in the HSQC spectrum to a proton resonance at δ 6.75 (d, J = 2.5 Hz; H-6′) that showed meta coupling in the COSY spectrum to the twinned proton resonances at δ 6.57 (d, J = 2.5 Hz; H-4′) and 6.55 (d, J = 2.5, Hz; H-4′). HMBC correlations observed between δ 6.75 (H-6′), 6.57 (H-4′), and 6.55 (H-4′) and carbon resonances at 146.0 (C-2′) and 151.2 (C-5′), in conjunction with the upfield shift of the proton resonances, were consistent with the presence of a hydroquinone ring in **1**. The existence of only meta coupling between the proton resonances assigned to the hydroquinone (H-6′ and H-4′) required that the sesquiterpenoid fragment (C-11) and one other substituent were both ortho to one of the phenol funtionalities.



An isolated ^1H spin system extending from the deshielded methine at δ 6.47 (H-1‴) to the geminal methylene protons at δ 3.75 (H-5‴) and 3.82 (H-5‴) was identified from the COSY, HSQC, and HMBC data. This spin system was assigned to a 2-deoxyfuranopentose fragment (C-1‴ to C-5‴) on the basis of the 2D NMR data. Subtracting the atoms present in the sesquiterpenoid ($C_{15}H_{25}$), hydroquinone ($C_6H_4O_2$), and 2-deoxyfuranose ($C_5H_9O_3$) fragments from the molecular formula of **1** showed that the remaining fragment had to account for $C_5H_2N_4O$ and 6 sites of unsaturation.

The ^1H resonances assigned to the remaining nitrogenous fragment all integrated for 0.5 protons each, were very deshielded (δ 8.37, H-8″; 8.36, H-8‴; 8.07, H-2″; 8.14, H-2‴), and were correlated in the HSQC spectrum to relatively deshielded carbon resonances (δ 8.37 and 8.36 to 141.0; 8.07 and 8.14 to 150.4 and 150.3, respectively), all suggestive of a purine substructure. HMBC correlations were

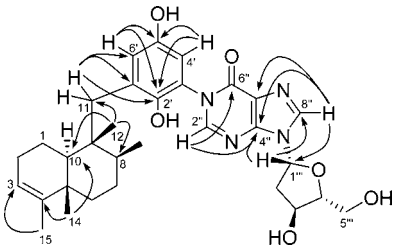


Figure 1. Selected HMBC correlations observed for **1**.

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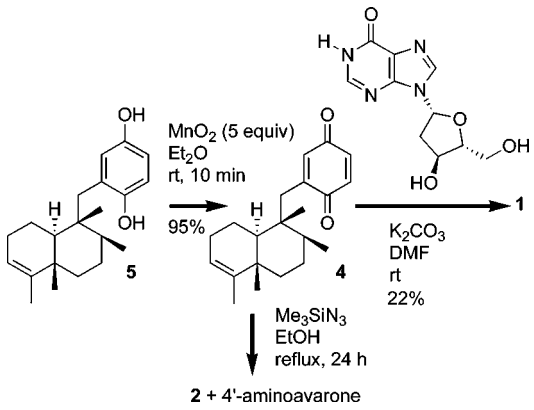
observed from the ^{1}H resonances at δ 8.36 and 8.37 (H-8″) to nonprotonated carbon resonances at δ 126.0 (C-5″) and 148.7 (C-4″) and to the furanose anomeric carbon at 86.6 (C-1‴), and from the furanose anomeric proton at δ 6.47 (H-1‴) to the nonprotonated carbon at 148.7 (C-4″) and the C-8″ carbon at 141.0, consistent with attachment of the deoxypentose fragment via the anomeric carbon C-1‴ to N-9″ of the purine, typical of a purine nucleoside. The remaining proton resonances assigned to the purine residue at δ 8.07 and 8.14 (H-2″) showed HMBC correlations to carbon resonances at δ 148.7 (C-4″) and 158.7 (C-6″), which completed the ^{13}C NMR assigment of the purine fragment. Comparison of the ^{13}C chemical shift assignments for this fragment to literature values for the purine residues of known oxygenated-purine nucleosides revealed a near identical resemblance to N-1 alkylated inosines.[5] This suggested that the constitution of avinosol (**1**) involved a bond between N-1″ of the purine fragment and C-3′ of the hydroquinone fragment.

2D NOESY correlations were observed between H-1‴ (δ 6.47) and both H-4‴ (δ 4.04) and H-2α‴ (δ 2.48), and between H-2β‴ (δ 2.77) and H-3‴ (δ 4.57), consistent with a ribose linked β to N-9″. The NOESY data also confirmed that the relative configurations in the sesquiterpenoid fragment were identical to those in avarol (**5**), thereby completing the proposed structure for **1** as shown. It is assumed that twinning of the proton resonances assigned to H-4′, H-2″ and H-8″, and several of the resonances assigned to carbon atoms in this region of the molecule, is due to the existence of atropisomers resulting from hindered rotation around the N1″-C-3′ bond. The H-4′ (δ 6.57 and 6.55) and H-2″ (δ 8.07 and 8.14) resonances show the greatest difference in chemical shifts in the two isomers, in agreement with this assumption. An absence of any twinning of the ^{1}H resonances assigned to the ribose fragment supports the N-1″ to C-3′ linkage rather than the alternative N-3″ to C-3′ linkage. Elevated temperature NMR experiments undertaken in an attempt to measure the magnitude of the N-3″/C-3′ rotational barrier led to decomposition of avinosol (**1**) before the coalescence temperature was reached.

To confirm the proposed structure of avinosol (**1**), the natural product was synthesized from avarone (**4**) and 2′-deoxyinosine as shown in Scheme 1. Avarone (**4**) was prepared in quantitative yield by oxidation of naturally occurring avarol (**5**),[6] obtained from the *Dysidea* sp. extract, with MnO_2 in Et_2O at room temperature for 10 min. Reaction of avarone (**4**) with 2′-deoxyinosine in DMF and K_2CO_3 at room temperature for 30 min gave avinosol (**1**) in 22% yield.[5] The synthetic material was identical to the natural product by TLC, NMR, and MS comparison, confirming the proposed constitution and the absolute configuration shown. Synthetic avinosol (**1**) was determined to have $[\alpha]^{22}_D = -26.9°$ (*c* 0.35, MeOH).

Avinosol (**1**) slowly oxidized to the corresponding quinone avinosone (**6**) upon exposure to air. There was no evidence for atropisomers in the NMR data obtained for **6**. This suggests that hydrogen bonding between the C-2′ phenol and the C-6″ carbonyl might play an important role in creating the barrier to rotation about the N-1″/C-3′ bond in avinosol (**1**).

Scheme 1. Synthesis of Avinosol (**1**) and 3′-Aminoavarone (**2**) from Avarol (**5**)



3′-Aminoavarone (**2**) was isolated as a red oil that gave a $[M + Na]^+$ ion at *m*/*z* 350.2097 in the HRESIMS appropriate for a molecular formula of $C_{21}H_{29}NO_2$ (calcd for $C_{21}H_{29}NO_2Na$, 350.2096). Analysis of the NMR data obtained for **2** (Supporting Information) readily identified the 3′ substituted avarone substructure found in the semisynthetic compound **6**. The molecular formula of **2** required that the 3′ substituent was an amino group. To confirm the structure of **2**, avarone (**4**) was reacted with Me_3SiN_3 in refluxing EtOH for 24 h to give a mixture of 3′-aminoavarone (**2**) and 4′-aminoavarone, that could be separated by HPLC.[7] The synthetic 3′-aminoavarone (**2**) was identical by TLC, NMR, and MS comparison with the natural product.

The 3′-phenethylaminoavarone analogue **3** was isolated as a red oil that gave a $[M + Na]^+$ ion at *m*/*z* 454.2726 in the HRESIMS consistent with a molecular formula of $C_{29}H_{37}NO_2$ (calcd for $C_{29}H_{37}NO_2Na$, 454.2722). The NMR data for **3** (Supporting Information) also contained resonances that could be assigned to the 3′-substituted avarone substructure found in avarone (**4**) and 3′-aminoavarone (**2**). An isolated ^{1}H spin system consisting of two scalar coupled methylene resonances (δ 2.93, t, J = 7 Hz, H-3″; 3.35, q, J = 7 Hz, H-2″) and a broad one proton resonance at δ 5.67 (NH-1″) was identified in the COSY data. The δ 5.67 resonance was correlated to the H-2″ resonance in the COSY spectrum but not correlated to a carbon resonance in the HSQC spectrum. A second isolated spin system in the ^{1}H NMR spectrum could be assigned to a monosubstituted phenyl ring (δ 7.21, d, J = 7.3 Hz, H-5″/H-9″; 7.24, t, J = 7.3 Hz, H-7″; 7.32, t, J = 7.3 Hz, H-6″/H-8″). In the HMBC spectrum, the H-2″ (δ 3.35) and H-3″ (δ 2.93) resonances were both correlated to the C-4″ resonance at δ 139.0, demonstrating that C-3″ was attached to the phenyl ring and, therefore, that N-1″ was attached to C-3′ of the avarone fragment as shown in **3**.

The pure meroterpenoids **1**, **2**, **4**, **5**, and **6** were tested in the antiinvasion assay against two human tumor cell lines

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with distinct modes of invasion. MDA-MB-231 breast cancer cells utilize the mesenchymal mode of invasion (path-generating) whereas LS174T colon carcinoma cells invade in an amoeboid manner (path-finding).[1] Avinosol (**1**) had an IC_{50} of ~50 μg/mL in the antiinvasion assay against both cell lines.[8] Avarone (**4**), avarol (**5**), and avinosone (**6**) were only active in the assay at concentrations of $\geq$100 μg/mL. 3′-Aminoavarone (**2**) did not show significant antiinvasion activity but did show differential cytotoxic effects on the cell lines used in the assay. It was found to be 10-fold more toxic to the MDA-MB-231 cell line (IC_{50} ~2 μg/mL) compared to the LS174T cell line (IC_{50} ~20 μg/mL). Further studies on the antiinvasive activity of avinosol (**1**) and the cytotoxic activity of 3′-aminoavarone (**2**) are ongoing in our laboratories.

There are a number of naturally occurring derivatives of avarone and other meroterpenoids where the aromatic/quinone fragment is substituted with simple amines and amino acids.[9] However, to the best of our knowledge, avinosol (**1**) is the first example of a natural product from any source that is a conjugate of a meroterpenoid and a nucleoside. The putative biogenesis of avinosol represents four distinct biosynthetic pathways: terpenoid, acetate/or shikimate, purine, and carbohydrate. It appears that nature has constructed avinosol (**1**) in an efficient convergent manner by first utilizing the common secondary metabolite pathway to the meroterpenoid fragment and a primary metabolite pathway to a nucleoside and then taking advantage of the intrinsic reactivities of the *p*-quinone in avarone and the purine ring in deoxyinosine to link them together.

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Supporting Information Available: Experimental section and tabulated NMR data and NMR spectra for avinosol (**1**), 3′-aminoavarone (**2**), 3′-phenethylaminoavarone (**3**), and avinosone (**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The meroterpenoid strongylophorine-26 has an IC_{50} of ~1 μg/mL in the same assay. See ref 2c.

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