Eleutherobin, a New Cytotoxin that Mimics Paclitaxel (Taxol) by Stabilizing Microtubules

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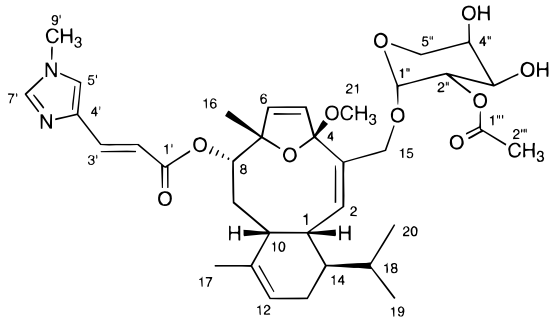
In connection with our long standing interest in the chemistry of marine soft-corals, we investigated a rare alcyonacean, identified as an *Eleutherobia* species (possibly *E. albiflora*, Alcyonacea, Alcyoniidea),[1] found near Bennett's Shoal in Western Australia. In the process of normal chemical examination, we isolated a new diterpene glycoside, eleutherobin (**1**), which possessed significant cytotoxicity against a wide variety



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of cancer cells. In subsequent testing, eleutherobin was shown to be a potent cancer cell inhibitor with an IC_{50} range of 10−15 nM *in vitro* against a diverse panel of tumor tissue cell lines.[2] More importantly, when investigated mechanistically, eleutherobin was found to stabilize microtubules by competing for the paclitaxel (Taxol) binding site on the microtubule polymer.[3,4] Evaluation of the stabilized microtubules by electron microscopy revealed a high degree of order indicative of this specific mechanism of action.[4] The tumor tissue selectivity of eleutherobin, determined in the National Cancer Institute's 60 cell panel, showed an approximate 100-fold increased potency (over the mean cytotoxicity) toward selected breast, renal, ovarian, and lung cancer cell lines. These selectivities are similar to those observed for paclitaxel, and indeed the NCI's COMPARE protocol identified paclitaxel as having a similar tumor-type selectivity (correlation coefficient 84%).

Eleutherobin (**1**) was isolated as a white noncrystalline solid from the dichloromethane−methanol extract of the lyophilized animal using conventional silica and reversed-phase (C-18) column and high performance chromatographic methods.[5] The purification of eleutherobin was easily monitored by following its cytotoxicity against HCT-116 human colon carcinoma *in vitro*. Eleutherobin was analyzed for $C_{35}H_{48}N_2O_{10}$ by HR-FABMS and NMR spectral methods, indicating 13 units of unsaturation.[4] Infrared and NMR data indicated the presence of two esters, one methoxy ketal, and one acetal, the latter being recognized as a component of a pentopyranose glycoside. The strong UV absorption of eleutherobin at 290 nm (log ϵ 3.82) was correlated by combined NMR methods to an *N*(1)-methylimidazole ring with added conjugation. Heterocorrelation NMR experiments (HMBC, HMQC) allowed the *N*-methylimidazole to be linked to the β carbon of an *(E)*-α,β-unsaturated ester, thus defining a *N*-methylurocanic acid ester. Comprehensive 2D NMR experiments, which included over 60 HMBC correlations, allowed all carbons and their respective protons to be assigned with confidence. These data lead to the assignment of the 4,7-oxaeunicellane skeleton to **1**.

The pentose sugar forming the glycoside was identified as 2″-*O*-acetyl-arabinose by a COSY NMR experiment in conjunction with coupling constant and chemical shift considerations. The acetate ester was placed at C-2″ based upon the lowfield proton and carbon shifts (^{1}H: δ 4.99; ^{13}C: 71.8 ppm) observed. Coupling constant analysis showed that the C-2″ and C-3″ protons were axial (J = 9.8 Hz), while those at C-1″ and C-4″ were equatorial. These data fully defined the glycoside as an α-2″-O-acetyl-arabinoside.

The relative stereochemistry of eleutherobin was readily established by NOESY NMR experiments. Correlations from the proton at C-10 to those at C-1 and C-8 established the *cis* ring juncture and placed the urocanate ester in the down position. An additional correlation from the C-10 proton to the C-20 methyl protons allowed the isopropyl group to be oriented on the top face of the molecule. Further correlations from the C-16 methyl protons to the C-8 proton and to the methoxy protons at C-4 established that these substituents were also on the top face of the molecule. Although the relative stereochemistry of the aglycon and sugar were independently established, no through space correlations were observed to correlate the stereochemistry of these two components. No absolute stereochemical information for eleutherobin has been obtained.

The aglycon of eleutherobin possesses the eunicellane carbon skeleton, a diterpene framework first observed in 1968 from a metabolite of the gorgonian octocoral *Eunicella stricta*.[6] Eunicellanes have subsequently been isolated from a wide variety of octocorals.[7] Eleutherobin is closely related to several other

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(1) Soft corals of the genus *Eleutherobia* (Alcyonacea, Octocorallia) are rare, but they have been reported from a variety of locales worldwide. We thank Dr. Phil Alderslade, Northern Territory Museum of Arts and Sciences, Darwin, Australia, for identifying this animal.

(2) The cytotoxic properties of eleutherobin, but not its tubulin stabilizing properties, were described in U.S. Letters Patent # 5,473,057 issued 5 December, 1995.

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(5) Eleutherobin (**1**), yield: 0.01% dry weight; $[\alpha]^{25}_D = -49.3°$ (*c* 3.0, MeOH); HRFABMS: $[M + Na]^+$ m/z = 679.3174, calc for $C_{35}H_{48}N_2O_{10}$-Na, 679.3207 (Δ −4.8 ppm); IR (NaCl, neat): 3360, 2960, 2922, 2856, 1722, 1657, 1450, 1372, 1243, 1152, 1055 cm^{-1}; UV (MeOH): λ_{max} (log ϵ) = 290 nm (3.824); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.96 (m, C-1), 5.56 (d, 9.2 Hz, C-2), 6.12 (d, 5.9 Hz, C-5), 6.09 (d, 5.9 Hz, C-6), 4.82 (d, 7.7 Hz, C-8), 1.39 (m, C-9), 1.61 (m, C-9), 2.61 (m, C-10), 5.28 (m, C-12), 1.98 (m, C-13), 2.32 (m, C-13), 1.23 (m, C-14), 3.88 (d, 12.4 Hz, C-15), 4.31 (d, 12.4 Hz. C-15), 1.45 (s, C-16), 1.52 (s, C-17), 1.57 (m, C-18), 0.97 (d, 6.5 Hz, C-19), 0.93 (d, 6.5 Hz, C-20), 3.22 (s, C-21), 6.57 (d, 15.5 Hz, C-2′), 7.55 (d, 15.5 Hz, C-3′), 7.10 (s, C-5′), 7.48 (s, C-7′), 3.72 (s, C-9′), 4.91 (d, 3.7 Hz, C-1″), 4.99 (dd, 9.8, 3.7 Hz, C-2″), 4.03 (dd, 9.8, 3.7 Hz, C-3″), 3.99 (m, C-4″), 3.70 (d, 11.9 Hz, C-5″), 3.83 (d, 11.9 Hz, C-5″), 2.11 (s, C-2‴); ^{13}C NMR (50 MHz, $CDCl_3$): δ 34.3 (CH, C-1), 137.4 (CH, C-2), 132.8 (C, C-3), 115.9 (C, C-4), 131.0 (CH, C-5), 133.7 (CH, C-6), 89.9 (C, C-7), 81.5 (CH, C-8), 31.5 (CH2, C-9), 38.7 (CH, C-10), 134.2 (C, C-11), 121.3 (CH, C-12), 24.5 (CH2, C-13), 42.4 (CH, C-14), 69.1 (CH2, C-15), 24.3 (CH3, C-16), 21.9 (CH3, C-17), 29.1 (CH, C-18), 20.5 (CH3, C-19), 22.2 (CH3, C-20), 49.6 (CH3, C-21), 166.7 (C, C-1′), 115.9 (CH, C-2′), 136.4 (CH, C-3′), 138.4 (C, C-4′), 122.9 (CH, C-5′), 139.5 (CH, C-7′), 33.6 (CH3, C-9′), 93.4 (CH, C-1″), 71.8 (CH, C-2″), 68.1 (CH, C-3″), 69.5 (CH, C-4″), 62.1 (CH2, C-5″), 171.4 (C, C-1‴), 21.0 (CH3, C-2‴).

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marine-derived eunicellanes, including the eleuthosides,[8] the valdivones,[9] and the sarcodictyins,[10] all of which possess the unique C-4−C-7 oxygen bridge. The eleuthosides and sarcodictyins also possess *N*(1)-methylurocanic acid esters at the C-8 position, and the eleuthosides possess analogous arabinose acetate substituents. Although no publications have appeared, a recent abstract reported the cytotoxicity of the sarcodictyins to be in the range of 400−900 nM, a value roughly 10-fold less potent than eleutherobin.[11] The same abstract reported that the sarcodictyins also possess paclitaxel-like microtubule-stabilizing properties.

When eleutherobin was first discovered[2] it was the second molecule to possess the unique microtubule stabilizing properties of paclitaxel. Since this time two other classes of compounds, the epothilones[12] and discodermolide,[13] have demonstrated similar properties. Whether eleutherobin, or the latter agents, will demonstrate the utility of taxol in treating cancer can only be answered after significant investment in the drug development and clinical trial processes.

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Supporting Information Available: ^{1}H NMR, UV, FT-IR, HMQC, ^{1}H COSY NMR, and ^{1}H NOESY NMR spectrum of eleutherobin (8 pages). See any current mastheadpage for ordering and Internet access instructions.

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