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HYDRALLMANOL A, AN INTERESTING DIPHENYL-p-MENTHANE DERIVATIVE OF MIXED BIOGENETIC ORIGIN FROM THE HYDROID *HYDRALLMANIA FALCATA*

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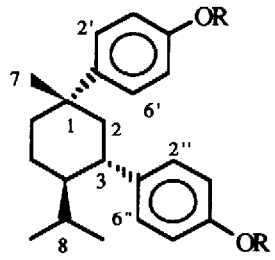
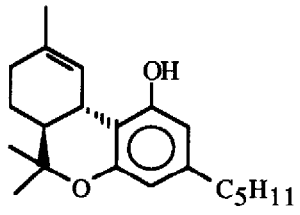
Summary *The structure of hydrallmanol A (1), a diphenyl-p-menthane derivative isolated from the marine hydroid* ***Hydrallmania falcata****, has been solved by spectroscopic analysis and confirmed via synthesis.*

Hydroids are small colonial marine invertebrates that belong to the phylum Cnidaria. Many species produce delicate upright colonies that resemble tiny bushes, trees, or feathers. Most hydroids are extremely difficult to collect in any abundance. As a consequence, very few chemical studies have been conducted on hydroids and it is not yet possible to specify a "typical" hydroid metabolite. Included in the small number of metabolites currently known from hydroids are a variety of polyhydroxylated steroids[1], a series of halogenated monoterpenes[2], a family of brominated β-carbolines[3], and a group of polyketide derived aromatic antibiotics.[4] We now wish to report the isolation of hydrallmanol A (**1**), a cytotoxic diphenyl-p-menthane derivative, from the North Atlantic hydroid *Hydrallmania falcata*.

Specimens of *H. falcata* were collected by hand using SCUBA at Rainy Cove, Nova Scotia. Freshly collected organisms were kept frozen until just prior to extraction. Thawed material was homogenized with methanol in a Waring blender and the methanol extract was concentrated *in vacuo*. The resulting residue was partitioned between water and dichloromethane. Fractionation of the dichloromethane soluble materials by sequential application of Sephadex LH 20 (1:1 MeOH/CH_2Cl_2), silica gel flash (2:8 EtOAc/CH_2Cl_2) and normal phase high performance liquid (1:9 EtOAc/CH_2Cl_2) chromatographies gave pure hydrallmanol A (**1**) as a pale yellow oil (3mg; 0.0004% wet weight).[5]





1 R=H
2 R=Ac

3

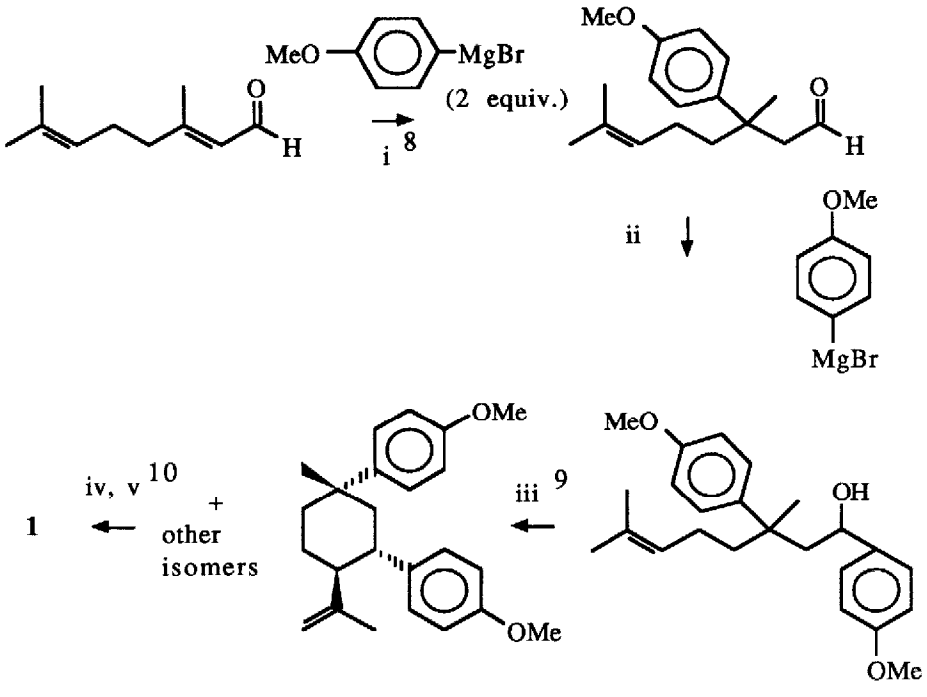
Hydrallmanol A (**1**) gave a parent ion at m/z 324.2097 daltons in the EIHRMS appropriate for a molecular formula of $C_{22}H_{28}O_2$ (ΔM +0.7mmu) requiring 9 degrees of unsaturation. The ^{1}H nmr spectrum of **1** (400MHz; $CDCl_3$) revealed a number of structural fragments. Four doublets at δ6.75 (J=8Hz, 2H), 6.76 (J=8Hz, 2H), 7.06 (J=8Hz, 2H) and 7.24 (J=8Hz, 2H) were assigned to the AA'XX' spin systems of two 1,4-disubstituted phenyl rings. Double resonance experiments showed that the doublet at δ 6.75 was coupled to the doublet at δ7.06 and that the doublet at δ 6.76 was coupled to the doublet at δ 7.24. The upfield chemical shift of the doublets at δ 6.75 and 6.76 implied that both phenyl rings were para substituted phenols. Treatment of **1** with acetic anhydride and pyridine gave the diacetate **2**.[6] The chemical shifts of the acetate methyl resonances in **2** (δ 2.27 and 2.28) verified the formation of phenolic esters.

A pair of methyl doublets at δ 0.74 (J=7Hz) and 0.83 (J=7Hz), which were both coupled to a methine resonance at δ 1.45-1.50, were also observed in the ^{1}H nmr spectrum of **1**. These resonances, in conjunction with an ion at m/z 281 (M^+ - C_3H_7) in the mass spectrum, identified the presence of an isopropyl fragment. A third methyl resonance at δ1.30 (s, 3H) was assigned to a tertiary methyl appendage. The two phenyl moieties in hydrallmanol A (**1**) accounted for 12 of the 22 carbon atoms, both of the oxygen atoms, and 8 of the 9 degrees of unsaturation. The remaining portion of the molecule consisted of a $C_{10}H_{18}$ fragment that had to contain one degree of unsaturation. There was no evidence in the spectral data of **1** or **2**[6] for the existence of an olefinic functionality apart from the phenyl rings. Therefore, the 10 carbon fragment had to be monocyclic and the presence of three methyl groups suggested a monoterpenoid substructure. Biogenetic considerations, in combination with the spectral data, guided the assembly of the identified fragments into the proposed diphenyl-p-menthane structure **1**.

Additional features of the ^{1}H nmr and mass spectral[7,9] data recorded for hydrallmanol A (**1**) supported the proposed structure. A deshielded methine resonance at δ 2.70 (ddd, J=12, 12, 4Hz) was assigned to $H3_{axial}$. Both double resonance and COSY

experiments showed that the H3 resonance was coupled to a pair of geminal methylene protons observed at δ1.70(bt, J=12Hz; $H2_{axial}$) and δ1.97(ddd, J=12, 4, 2Hz; $H2_{equatorial}$) and to a methine proton observed at δ1.45(m; $H4_{axial}$). The 2 Hz coupling observed in the $H2_{equatorial}$ resonance was assigned to a W coupling with $H6_{equatorial}$. The absence of additional scalar couplings into the $H2_{equatorial}$ and $H2_{axial}$ resonances was consistent with the presence of a quaternary center at C1. Irradiation of H3 (δ2.70) induced nOe's into the methyl singlet at δ 1.30 (Me7) and into the aromatic doublet at δ 7.06 (H2" & H6"), while irradiation of the Me7 resonance at δ 1.30 induced nOe's into H3 (δ2.70) and the aromatic doublet at δ7.24 (H2' & H6'). The nOe experiments, in conjunction with the coupling constants observed in the H3 resonance, allowed a complete assignment of the relative stereochemistry of **1** as shown, and they also facilitated the assignment of the aromatic resonances to specific hydrogen atoms as indicated above.

SCHEME I



i) CuI, THF, 17hr; ii) THF, RT, 6hr; iii) p-TsOH, chloroform, reflux 7hr; iv) hydrogen, Pd/C, EtOAc, RT, 24hr; v) Me_3SiI, CH_3CN, reflux, 40hr

Hydrallmanol A (**1**) was synthesized (See Scheme I) in order to verify the proposed structure and to provide sufficient material for biological testing. Synthetic **1** was identical to the natural material by tlc, ^{1}H nmr, ms and ir comparison. The racemic material obtained via synthesis exhibited mild cytotoxicity in the L1210 assay (ED_{50} 30μg/mL).[11]

Phenyl substituted p-menthanes are well documented from terrestrial plants. Δ^1-3,4-*trans*Tetrahydrocannabinol (**3**), isolated from *Cannabis sativa*, is one of the best known examples.[12] Hydrallmanol A (**1**) represents the first example of a phenyl-p-menthane from an animal source, the first example from any marine organism, and it is apparently the first example, from any source, in which the menthane skeleton contains two phenyl substituents.

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5. A number of closely related compounds were also isolated, however, they were obtained in such small quantities that it was impossible to determine their structures.

6. Diacetate **2** shows: ^{1}H nmr (400MHz, $CDCl_3$) δ 0.75(d, J=7Hz), 0.83(d,J=7Hz), 1.33(s), 2.01(s), 2.27(s), 2.78(ddd,J=12,12,4Hz), 6.99(d,J=8Hz), 7.01(d,J=8Hz), 7.18(d,J=8Hz), 7.36(d,J=8Hz); ^{13}C nmr (75MHz, $CDCl_3$) δ15.3, 20.3, 21.1, 21.4, 25.0, 27.2, 29.7, 37.3, 43.1, 47.5, 47.8, 49.2, 120.6, 120.8, 120.9, 121.3, 127.2, 128.5, 147.0, 149.2, 170.0, 179.0.

7. In the EIMS, hydrallmanol A (1) shows: m/z(rel. intensity %) 324 M^+(15), 309(6), 239(9), 189(6), 147(20), 135(73), 121(26), 107(100), 43(11).

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