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STYPOTRIOL AND STYPOLDIONE; ICHTHYOTOXINS OF MIXED BIOGENESIS FROM THE MARINE ALGA Stypopodium zonale[1]

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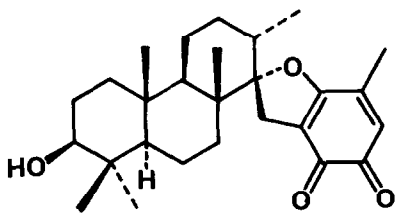
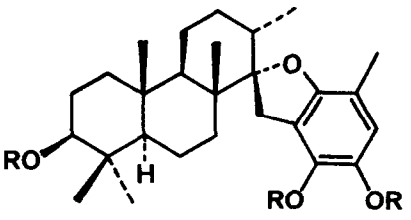
The tropical brown alga Stypopodium zonale (Lamouroux) Papenfuss[3] (Dictyotaceae, Phaeophyta) grows luxuriantly, frequently to 20 cm in height, in the western Caribbean where herbivore pressure is very intense. When fresh S. zonale is placed in an aquarium, the water soon turns a rust color, and water conditioned in this fashion is rendered extremely toxic to the reef-dwelling herbivorous damsel fish Eupomacentrus leucostictus. The fish immediately senses the toxin and attempts to jump out of the aquarium. This behavior is followed by erratic responses to external stimuli, apparent difficulty in obtaining oxygen, loss of equilibrium, narcosis, and eventually death. Diethyl ether extraction of the water yields a complex mixture of UV-absorbing compounds, the major component of which is the bright red ortho-quinone, stypoldione (1). Pure 1, redissolved in seawater (1.0 μg/ml), induced the same toxic symptoms as noted for the algae-conditioned seawater.[4]

The crude $CHCl_3$/MeOH extract of S. zonale was found to be even more toxic than 1, indicating the existence of an additional and more potent intracellular toxin. Silica gel column chromatography of the crude extract yielded 1 as the major toxic component, but when the extract was acetylated (Ac_2O/py/25°) prior to column chromatography, only stypotriol triacetate (3) could be isolated. $LiAlH_4$ reduction of 3 yielded stypotriol (2), which, although rapidly air-oxidized to the quinone 1, was found to be stable in the crude extract, and indefinitely stable when stored in the presence of hydrogen with a trace of platinum catalyst. The greater toxicity of stypotriol (LD $\cong$ 0.2 μg/ml) accounts for the enhanced toxicity of the crude extract.



1



2 R = H
3 R = Ac

Crystallization of stypoldione (1) from diethyl ether/methanol gave crystals, mp 170° dec., suitable for spectral and X-ray diffraction studies. The ortho-quinone 1, $[\alpha]_D^{25}$ -65.1° (c 4.61, $CHCl_3$), isolated as 6% of the crude extract, showed UV and IR absorptions for the o-quinone moiety ($\nu_{C=0}$ = 1675, 1650 cm^{-1}; λ_{max}^{MeOH} = 475, 270 nm, ε = 882, 2470), and IR absorptions which confirmed the presence of the hydroxyl group (ν_{O-H} = 3500 cm^{-1}). The mass spectrum of 1 showed a parent ion at m/e = 426 ($C_{27}H_{38}O_4$) and an M^+ + 2 peak at m/e = 428, characteristic of the quinone-hydroquinone reduction which frequently occurs in the mass spectrometer.[5] The ^1H NMR spectrum of stypoldione (220 MHz, CCl_4) consists of the following bands: δ 5.99 (1H, m, J = 1 Hz), 3.11 (1H, dd, J = 10,4 Hz), 2.90 (1H, d, J = 16 Hz), 2.59 (1H, d, J = 16 Hz), 2.14 (3H, d, J = 1.0 Hz), 1.2 - 1.9 (15H, m), 0.97 (3H, s), 0.96 (3H, s), 0.85 (3H, s), 0.73 (3H, s), 0.74 (3H, d, J = 6 Hz). These combined spectral features, taken with biogenetic considerations, suggested the formulation of these metabolites as structures 1 - 3.

Stypotriol (2), $[\alpha]_D^{25}$ - 10.0° (c 0.82, $CHCl_3$), was obtained either by rapid column chromatography of the extract[6], or by catalytic reduction (H_2/Pt) or $Na_2S_2O_4$ reduction[7] of stypoldione. The triol showed only hydroxyl absorptions in its IR spectrum, and the following ^1H NMR bands: (220 MHz, $CDCl_3$) δ 6.44 (1H, s), 5.67 (1H, D_2O exchangeable), 5.65 (1H, D_2O exchangeable), 3.24 (1H, dd, J = 10,4 Hz). 3.18 (1H, d, J = 16 Hz), 2.80 (1H, D_2O exchangeable), 2.76 (1H, d, J = 16 Hz), 2.10 (3H, s), 0.97 (3H, s), 0.92 (1H, s), 0.85 (3H, s), 0.76 (3H, s), 0.68 (3H, d, J = 6 Hz).

Stypotriol triacetate (3), mp 248-50°, was obtained from both the direct acetylation of 2 and from column chromatography of the pre-acetylated extract. The triacetate showed ester carbonyl infrared absorptions at 1735 cm^{-1}, and ^1H NMR bands at δ 2.11 (6H, s), and 1.92 (3H, s), characteristic of the three acetate methyl groups.

The structure of 1 was rigorously established by X-ray crystallography. Stypoldione (1) crystallized in the monoclinic crystal class as bright red cubes.

Cell constants, determined by a least squares fit of 15 moderate 2θ values (35-45°), were $\underset{\sim}{a}$ = 11.857(6), $\underset{\sim}{b}$ = 7.386(4), $\underset{\sim}{c}$ = 15.548(7) Å and $\underset{\sim}{\beta}$ = 108.74(6)°. Systematic extinctions uniquely designated the chiral space group $P2_1$. A calculated density (z=2) of ~1.10 g/cc indicated one molecule of $C_{27}H_{38}O_4$ formed the asymmetric unit although the measured density was always slightly higher. Intensity data were collected on a fully automated four circle diffractometer with graphite monochromated CuKα (1.54178 Å) radiation. A variable speed w-scan technique was employed and all unique diffraction maxima with $2\theta \leq 114.1°$ (.92 Å) were surveyed. After correction for Lorenz, polarization and background effects, 1414 (70%) of the 2008 reflections were judged observed [$I \geq 3\sigma(I)$]. A phasing model was reached uneventfully through the application of a multisolution, weighted tangent formula approach[8] followed by recycling plausible fragments through tangent formula refinement.[9] Hydrogen atoms were located on difference electron density syntheses. The difference maps also indicated that there was additional high electron density not accounted for by our model of stypoldione which we attribute to a solvent of crystallization. This residual electron density is modeled by three independent partial atoms which form an infinite chain around the screw axis at 1/2, 0, 1/2. This chain has alternating short (~1.36 Å) and long (~1.56 Å) bonds and we believe that it is a badly disordered methanol which was one of the crystallization solvents. Full matrix least squares refinement with anisotropic nonhydrogen atoms and isotropic hydrogens converged to a conventional crystallographic residual of 0.087 for the observed data.[10]

Only the relative configuration of stypoldione (1) was determined by the X-ray experiment, and no absolute configuration is implied as drawn in structure 1. The three cyclohexane rings are all in the chair conformation and are joined in a trans, anti, trans fashion. All bond distances and angles generally agree with expected values and no unusually large peaks were found in the final difference synthesis. There is an intermolecular hydrogen bond between the hydroxyl group and the quinone carbonyl group para-substituted to the aromatic methyl.

Stypoldione and stypotriol are closely related to the algal metabolite taondiol, isolated from the taxonomically related brown seaweed Taonia atomaria.[11] Stypopodium zonale does not produce taondiol, but this toxic alga does produce several other stypotriol-related compounds which are currently under investigation.

Acknowledgments

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