Vidalenolone, a Novel Phenolic Metabolite from the Tropical Red Alga *Vidalia* sp.

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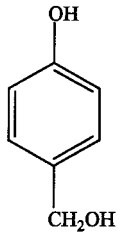
The Indonesian red alga *Vidalia* sp. was identified as a candidate for fractionation because its crude lipid extract showed activity in a mechanism-based anticancer assay (Fyn SH2-inhibitory activity). A chemically novel phenolic metabolite, vidalenolone, as well as two previously described and structurally simple phenols, were isolated as SH2-inactive substances. Their structures were determined by an interplay of spectroscopic methods, principally 2D NMR, and reference to literature data.

Emerging targets in the potential treatment of proliferative disorders, including cancer, are the intracellular signal transduction pathways that connect cell surface receptors for growth stimulatory molecules with gene-activating pathways in the cell nucleus.[1] One of the more common protein−protein interactions that mediates signal transduction is SH2 domain binding, a process that involves a common motif for recognition and a resulting transfer of a phosphate group to a tyrosine phenol group.[2] As many forms of cancer are inappropriately regulated in these pathways, they have become a promising theoretical target for new cancer chemotherapeutics.[3] We report here the detection of SH2 inhibitory activity in the organic extract of a tropical red alga, *Vidalia* sp., and the subsequent isolation of two known substances (**1**, **2**) and one structurally novel metabolite, vidalenolone (**3**), all of which were inactive in the SH2 inhibitory assay.



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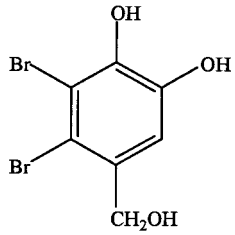

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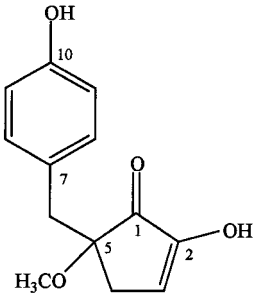

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Vidalia spp. occur tropically and have previously been a source of antiinflammatory bromophenols,[4] a brominated cyclopentenone derivative,[5] and an unusual amino acid.[6] Collected as part of our survey of the biologically active compounds from Indonesian seaweeds, the alga afforded an extract that substantially inhibited SH2 interactions between Fyn-type proteins. Isolation of vidalenolone (**3**) and of the simple phenols *p*-hydroxybenzyl alcohol (**1**) and ethyl lanosol (**2**) was accomplished using the in vitro assay to direct the process; however, at an intermediate stage in the isolation process, all fractions were found inactive in the SH2 assay.

A subtidal collection of the tropical red alga *Vidalia* sp. was obtained from −20 to −30 m by scuba at Ang Island, Indonesia, on November 5, 1994. It was preserved in *i*-PrOH at low temperature until extraction with $CHCl_3$/MeOH (2:1), which yielded 6.0 g of an oily tar. The extract was evaluated for inhibitory activity to a suite of SH2-domain interacting proteins, including Fyn, Abl, Crk, and Grb2, and found to be selective for Fyn (>50% inhibition at 20 μg/mL). Assay to these targets was used to direct the repetitive fractionation by silica gel column chromatography and then HPLC (see Experimental Section) to yield three pure compounds, *p*-hydroxybenzyl alcohol (**1**), ethyl lanosol (**2**), and vidalenolone (**3**). Unfortunately, none of the three were active as inhibitors of Fyn or any other SH2 protein interaction.

Vidalenolone (**3**) was isolated as an optically active oil, $[\alpha]^{25}_D$ −95° (*c* 0.31, MeOH), which had a complex UV spectrum suggesting the presence of conjugated enone and phenol chromophores. The molecular formula of vidalenolone was provided by HR CIMS (positive ion), which gave an $[M + H]^+$ at 235.0971, which analyzed for $C_{13}H_{15}O_4$. The seven degrees of unsaturation in this formula could be partially accounted for by one carbonyl and four double bonds; hence, compound **3** was bicyclic.

Three partial structures could be constructed that fully accounted for all of the atoms in vidalenolone (Figure 1). A *p*-hydroxybenzyl moiety (**A**) was apparent from ^{1}H NMR data which showed two 2H coupled doublets at δ 6.90 and 6.60 as well as a 2H AB pattern at δ 2.73/2.67, ^{13}C NMR data which showed four characteristic aromatic bands [δ 156.0, 131.1 (2C), 125.6 (1C), 114.7 (2C)] and a mid-field methylene at δ 40.7, and CI MS data which showed a major fragment peak at *m*/*z* 107 for C_7H_7O. Additionally, a phenolic hydroxyl group was apparent from both IR (3450 cm^{-1}) and ^{1}H NMR (δ 9.25) data. A second partial structure (**B**) was defined by HMBC wherein a methoxy group (^{1}H δ 3.05, ^{13}C δ 51.0) showed long-range coupling to a deshielded quaternary carbon (δ 80.4). A third partial structure (**C**) consisted of an enone as suggested from absorptions in the

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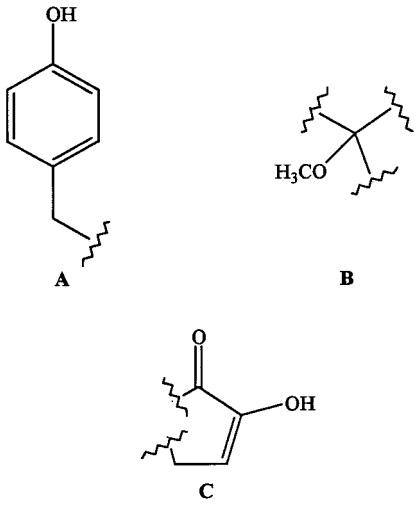

Figure 1. Partial structures **A**, **B**, and **C** of vidalenolone (**3**).

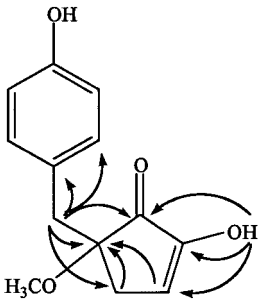

Figure 2. Key HMBC correlations used to connect partial structures **A**, **B**, and **C** of vidalenolone (**3**).

IR ($\nu_{C=O}$ 1700 cm^{-1}) and ^{13}C NMR for a conjugated carbonyl (δ202.8) and polarized double bond (δ 153.2, 129.7). The higher field of these two vinyl carbons was associated with a proton at δ 6.30, which in turn was coupled by 3.1 Hz to an allylic methylene (^{1}H δ 2.42; ^{13}C δ 30.2). The presence of a vinyl hydroxyl group attached to the α-carbon of the enone, confirmed by HMBC correlations between its hydrogen-bonded proton at δ 9.55 and all three carbons of the enone, explained the unusual polarization of this constellation.

Connection between these three partial structures was made by interpretation of HMBC data (Figure 2). Correlations between the C-6 methylene hydrogens at δ 2.73/2.69 and the quaternary carbon C-5 connected partial structure **A** and **B**. Similarly, HMBC correlations between the H-3 vinyl proton/H-4 methylene protons and C-5 connected one portion of partial structure **C** with partial structure **B**. The final crucial link between partial structure **B** and **C** was an HMBC correlation between the H-6 methylene protons and the C-1 carbonyl, completing the planar structure of vidalenolone (**3**). The absolute stereochemistry at C-5 remains to be determined.

Vidalenolone is a biosynthetically intriguing structure, as it is uncertain if a tyrosine-derived unit condenses with two malonyl CoA units and then decarboxylates, cyclizes, and is adjusted in oxidation state (Figure 3a) or if a tyrosine unit first condenses with a succinate-like unit and then undergoes similar transformations to form metabolite **3** (Figure 3b).

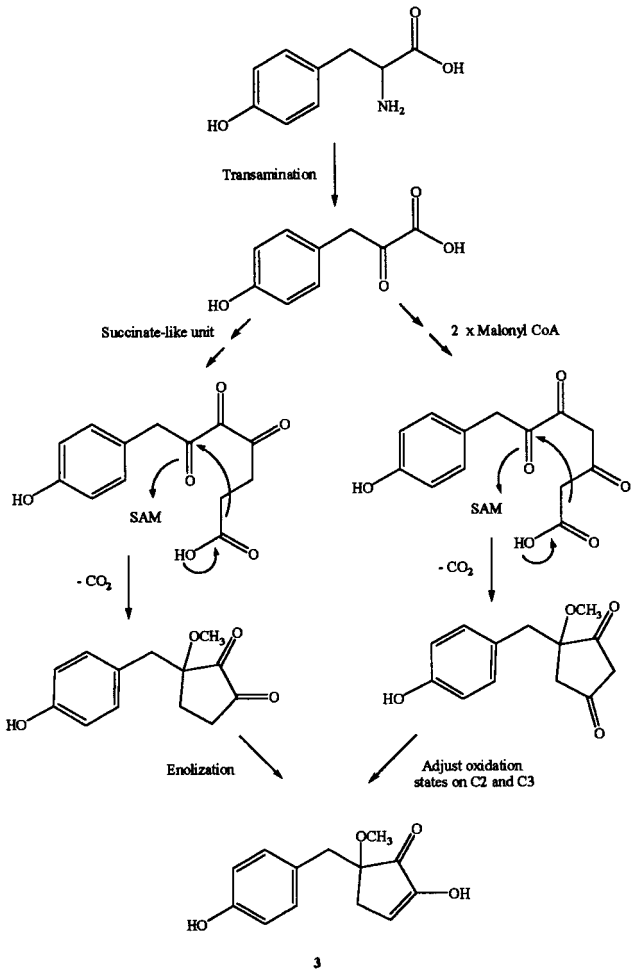

Figure 3. Alternate biogenetic pathways to vidalenolone (**3**) involving a tyrosine-derived subunit that is extended by either (a) two malonyl CoA units or (b) a succinate-like unit.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer and IR spectra on a Nicolet 510 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. NMR spectra were determined on a Bruker AM 400 spectrometer operating at a frequency of 400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C spectra or on a Bruker ACP 300 spectrometer operating at a frequency of 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C acquisitions. Chemical shifts are reported relative to a tetramethylsilane internal standard (δ), and coupling constants are reported in Hz. Low-resolution mass spectra (LRMS) were obtained on a Finnigan 4023, while high-resolution mass spectra (HRMS) were performed on a Kratos MS 50 TC spectrometer. Waters equipment, including M6000A and M45 pumps, U6K injector, R 401 differential refractometer, and Lambda-Max Model 480 variable wavelength detectors were employed for HPLC. Thin-layer chromatography was performed using Merck TLC sheets with fluorescent indicator (Si gel 60 F_{254}). Chromatographic solvents were HPLC-grade or distilled from glass prior to use.

Plant Materials. The algal material, *Vidalia* sp., was growing on volcanic substrate at −20 to −25 m at Ang Island off the No. Coast of Sulawesi, Indonesia, on November 5, 1994. A voucher specimen is available from WHG as IAI-5Nov94-1.

Extraction. The freshly collected material (3 L compressed volume) was preserved in *i*-PrOH at low temperature until 3-fold extraction at ambient temperature with $CHCl_3$/MeOH (2:1) and homogenization (559 g dried algal mark). The extraction solvent was removed in vacuo to yield 6.02 g of a dark green oil.

Isolation. A major portion of the extract (4.9 g) was applied to a Si gel vacuum liquid column and fractionated using a stepped gradient of hexanes/EtOAc. A portion (490 mg) of the

fractions eluting with 80% EtOAc/hexanes which continued to show Fyn-inhibitory activity (515 mg) was further chromatographed over a Si gel column to yield eight fractions. None of these latter fractions were active to any of the SH2 protein targets. Material eluting in 10% EtOAc/hexanes (164 mg) was further purified by HPLC (2 × 300 mm × 4.1 mm Versapack Si 10 μm columns, 15% EtOAc/hexanes) to give *p*-hydroxybenzyl alcohol (**1**, 14 mg) by comparison with literature data.[7] Material eluting with 50% EtOAc/hexanes (129 mg) was predominately ethyl lanosol (**2**) and was identified by comparison of its ^{1}H NMR spectrum with literature values.[8] A subsequent fraction also eluting with 50% EtOAc/hexanes (40 mg) was repetitively purified by HPLC (2 × 300 mm × 4.1 mm Versapack Si 10 μm columns, 25% EtOAc/hexanes) to give pure vidalenolone (**3**, 2.0 mg).

Vidalenolone (3): colorless oil; $[\alpha]^{25}_D$ −95° (*c* 0.31, MeOH); CD (MeOH): $\Delta\epsilon$ −3.3, −1.3, −1.8 (λ_{max} 220, 250, 270 nm); UV λ_{max} 209, 224, 266 (log ϵ 4.50, 4.54, 4.08); IR (film) ν 3450 (br), 2935, 2335, 1700, 1625 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ 9.55 (1H, *H*O−), 9.25 (1H, *H*O−Ph−), 6.90 (2H, d, J = 8.3 Hz, H-8/H-12), 6.60 (2H, d, J = 8.3 Hz, H-9/H-11), 6.30 (1H, t, J = 3.1, H-3), 3.05 (3H, s, −OCH$_3$), 2.73, (1H, d, J = 13.0 Hz, H-6a), 2.67 (1H, d, J = 13.0 Hz, H-6b), 2.42 (2H, m, H$_2$-4); ^{1}H NMR (CDCl$_3$, 300.13 MHz) δ 7.02 (2H, d, J = 8.0 Hz, H-8/H-12), 6.71 (2H, d, J = 8.0 Hz, H-9/H-11), 6.40 (1H, t, J = 3.0 Hz, H-3), 3.24 (3H, s, −OCH$_3$), 2.97 (1H, d, J = 13.0 Hz, H-6a), 2.88 (1H, d, J = 13.0 Hz, H-6b), 2.62 (1H, dd, J = 17.0, 3.0 Hz, H-4a), 2.53 (1H, dd, J = 17.0, 3.0 Hz, H-4b); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ 202.8 (C-1), 156.0 (C-10), 153.2 (C-2), 131.1 (C-8/12), 129.7 (C-3), 125.6 (C-7), 114.7 (C-9/11), 80.4 (C-5), 51.0 (−OCH$_3$), 40.7 (C-6), 30.2 (C-4); LR CIMS (pos. ion, rel int) obsd [M + H]$^+$ at *m*/*z* 235 (18), 234 (13), 219 (2), 217 (2), 205 (18), 204 (21), 203 (100), 157 (6), 135 (6), 129 (45), 128 (90), 107 (78); HR CIMS (pos. ion, rel int) obsd [M + H]$^+$ at *m*/*z* 235.0971 (+0.1 mmu error, calcd for $C_{13}H_{15}O_4$, 235.0970).

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Supporting Information Available: Methods for expression of recombinant proteins and the SH2 ELISA. This material is free of charge via the Internet at http://pubs.acs.org.

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