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Dysidiolide: A Novel Protein Phosphatase Inhibitor from the Caribbean Sponge *Dysidea etheria* de Laubenfels

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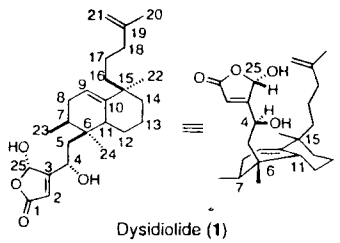
Marine sponges continue to be a rich source of secondary metabolites with novel structures and desirable biological activity.[1] In a continuing search for new protein phosphatase inhibitors from marine organisms, we have isolated a novel cdc25A inhibitor from the marine sponge *Dysidea etheria* de Laubenfels. The inhibitor, trivially named dysidiolide, is a sesterterpene γ-hydroxybutenolide, and the structure, which represents an unusual cyclization of a C_{25} isoprenoid, was determined by single-crystal X-ray diffraction.

The cdc25 protein phosphatase has been shown to activate the G_2/M transition of the cell cycle by the dephosphorylation of the $p34^{cdc2}$/cyclin B complex at both the Tyr-15 and Thr-14 residues.[2] Cdc25 exists as three human homologs termed cdc25A, -B and -C, the precise functions of which have yet to be fully described. The cdc25A homolog has been cloned and expressed as a GST conjugate in *Escherichia coli*, and a screening system for inhibitors of this enzyme has been described.[3] It is thought that inhibitors of this enzyme will be able to block the cell cycle at the G_2/M transition point and produce cell cycle arrest. This antimitotic activity may have utility in the treatment of cancer and other proliferative disorders.

The sponge specimen (HBOI No. 26-V-93-4-003) was collected by scuba diving at a depth of 60 ft off Long Island, Bahamas, and was stored at −20 °C until extraction. The specimen was soaked in EtOH and the concentrated EtOH extract partitioned between BuOH and H_2O. The BuOH soluble fraction was chromatographed over silica gel with CH_2Cl_2/MeOH, and fractions were monitored for inhibition of the cdc25A protein phosphatase. The fraction that showed the greatest inhibition of cdc25A was further purified by reversed-phase HPLC with aqueous MeOH (22.5% H_2O) to give pure dysidiolide (**1**). Crystallization of dysidiolide from 2% MeOH/



Dysidiolide (**1**)

CH_2Cl_2 yielded colorless crystals (yield 0.05% of wet weight), mp 186−187 °C, $[\alpha]^{24}_D$ −11.1° (*c* 0.6, CH_2Cl_2/CH_3OH, 1:1). High-resolution FABMS, *m/z* confirmed the formula $C_{25}H_{38}O_4$ [$(M + H^+)$ *m/z* 403.2531, Δ 0.4 mmu]. The UV spectrum gave only an end absorption, and the IR spectrum showed absorptions at 3335 and 1741 cm^{-1} compatible with hydroxyl and α,β-unsaturated γ-lactone groups, respectively.[4] The ^1H NMR spectrum revealed the presence of a secondary methyl, a vinyl methyl, two quaternary methyls, a hydroxy methine, two vinylic methines, a vinylic methylene, and several overlapping methylene groups. Further, due to possible isomerization of the semialdehyde group in the γ-hydroxybutenolide functionality, the ^1H NMR spectrum[5] indicated doubled ^1H signals for the hydroxy methine and hydroxyl protons. One of the quaternary methyls was broad and thus tentatively assigned its chemical shift value. The ^{13}C NMR spectrum[6] in DMSO-d_6 indicated a set of sharp signals[7] and a set of very broad signals. Sharp ^{13}C signals were observed for all four methyls, three of eight methylenes, a single terminal methylene, one of two vinyl methines, three of four methines, and two of five quaternary carbons. Some of these broad signals were hardly visible over the noise level. In addition, the ^{13}C signals constituting the γ-hydroxybutenolide group and two carbon atoms down the chain appeared doubled, further complicating the spectrum of this compound. Since no unequivocal structure could be determined from these data, the compound was crystallized for X-ray studies.

The relative stereostructure of dysidiolide was determined by single-crystal X-ray diffraction. Dysidiolide crystallizes in space group $P2_12_12_1$ with a = 7.377(1) Å, b = 10.189(1) Å, and c = 30.352(3) Å and refined to a conventional R factor of 5.9% for the 1562 reflections with $|F_o| \geq 4\sigma(F_o)$. The X-ray experiment defined only the relative, not the absolute, stereochemistry, so the enantiomer shown is arbitrary. As can be seen in the final X-ray model shown in Figure 1, the molecular conformation has all methyl groups in equatorial or pseudoequatorial positions, and the two large substituents occupy axial and pseudoaxial positions on the same side of the molecule. This diaxial conformation and the extended structure for both chains lead to the projection of two parallel arms projecting from the β-face of dysidiolide. Since the two arms are rather close, little over 4 Å apart, it is possible that hindered motions of the γ-hydroxybutenolide give rise to the multiple peaks seen in the NMR spectrum. Since it is likely that the γ-hydroxybutenolide moiety serves as a surrogate phosphate when dysidiolide is bound to cdc25A, the long side chain may occupy a hydrophobic binding pocket associated with the normal substrate. However, we do not currently have evidence to support this hypothesis.

Dysidiolide has a novel carbon skeleton with unique structural features that have not been previously encountered in a natural product. (+)- Dysideapalaunic acid[8] isolated from another *Dysidea* sp. collected from the Palauan Sea has been reported to possess a similar bicyclic arrangement; however, the carbon skeleton differs from that of dysidiolide. Several other β-alkyl-γ-hydroxybutenolide terpenoids have been isolated from marine

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(4) UV (MeOH) 203 nm (ε 19 000); IR (KBr) 3335, 2916, 1741, 1649, 1447, 1275, 1247, 1131, 1076, and 947 cm^{-1}.

(5) ^1H NMR (500 MHz, DMSO-d_6) δ 0.81 (3H, d, J = 6.7 Hz, H-23), 0.93 (3H, s, H-22), 1.51(3H, br s, H-24) 1.62 (3H, s, H-20), 4.38, (4.51) (1H, dt, J = 5.7, 8.6 Hz, H-4), 4.60, 4.63 (2H, 2s, H-21), 5.12, (5.23) (1H, d, J = 5.7 Hz, 4-OH), 5.91 (1H, s, H-2), 6.08 (1H, d, J = 6.0 Hz, H-25), 7.80 (1H, d, J = 6.0 Hz, 25-OH).

(6) ^{13}C NMR (125.7 MHz, DMSO-d_6) δ 14.9 (q, C-23), 21.5 (br), 21.6 (t), 21.7 (t), 22.0 (q, C-20), 22.0 (q, C-24), 23.5 (br), 25.9 (q, C-22), 27.0 (br), 29.8 (br), 31.0 (br), 33.0 (d, C-7), 36.6 (br), 37.9 (t), 39.0 (br), 41.0 (br), 63.0, (64.4) (d, C-4), 97.6, (98.1) (d, C-25), 110.0 (t, C-21), 115.5 (d, C-9), 115.9, (116.2) (d, C-2), 142.3 (s, C-10), 145.3 (s, C-19), 170.4, (170.5) (s, C-1), 173.5, (175.5) (s, C-3).

(7) Results from HMQC and HMBC NMR experiments were used to assign the respective carbon signals.

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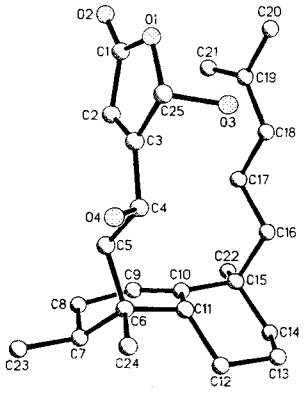

Figure 1. A computer-generated perspective drawing of the X-ray model of dysidiolide (**1**). The absolute configuration shown is arbitrary.

organisms. These include mokupalides,[9] manoalides,[10] luffariellins,[11] luffariellolide,[12] palauolide,[13] cacospongionolide,[14] aplysolides,[15] aplyolide A,[15] and luffarins.[16] Dysidiolide is the first compound reported with this substructure from the genus *Dysidea*.

Dysidiolide inhibited the dephosphorylation of *p*-nitrophenol phosphate by cdc25A with an IC_{50} of 9.4 μM.[17] Dysidiolide showed no inhibition of the protein phosphatases calcineurin, CD45, and LAR when tested at 12.4 μM. Dysidiolide inhibited growth of the A-549 human lung carcinoma and P388 murine leukemia cell lines with IC_{50} values of 4.7 and 1.5 μM, respectively. To the best of our knowledge, this is the first report of a cdc25A active compound isolated from a natural source. Further studies are needed to determine whether the inhibition of cell growth by dysidiolide is due to the inhibition of cdc25A observed *in vitro*.

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Supporting Information Available: ^1H and ^{13}C NMR spectra and X-ray data (11 pages). See any current masthead page for ordering and Internet access instructions.

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