

ISOLATION AND IDENTIFICATION OF A NEW CEMBRANOID
DITERPENE FROM THE TUNICATE *STYELA PLICATA*

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ABSTRACT.—We report the isolation of a novel cembrane, diterpene styelolide [**1**], from the tunicate *Styela plicata*. The structure of the new diterpene was determined by utilizing 2D nmr techniques.

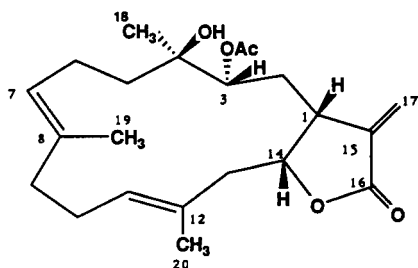
In the course of our continuing search for terpenoids from marine invertebrates, we have isolated a new cembranoid diterpene from the tunicate *Styela plicata* (Leseur) (Class Ascidiacea, order Stolidobranchiata). Previous studies of soft corals and mollusks that graze on soft coral have revealed an abundance of cembrane derivatives (1–3). We report the first isolation and structure of a novel cembrane, styelolide [**1**], from a tunicate. Because the occurrence of a cembranolide in a tunicate is unprecedented, a thorough examination of the specimen was made to determine the presence of contaminating invertebrates. The collected specimens were devoid of any visible contaminant (with the exception of a nylon rope) thus eliminating the possibility of the origin of the isolated cembranoid in a contaminating soft coral.

The tunicate, collected in northwestern Florida, was extracted with *i*PrOH. The residue from the *i*PrOH extract was subjected to solvent partitioning followed by repeated cc. Final purification was accomplished by hplc.

Examination of the ^{13}C -nmr spectrum (75 MHz, CDCl_3) revealed the

presence of 22 carbons including two ester carbonyl carbons (δ 170.5, 167.3), three oxygenated (δ 82.3, 74.1, 73.4), and six olefinic (resonating between δ 137 and 125) carbons. An attached proton test (APT) (4) provided multiplicities of all 22 carbons, indicating that two of the three oxygenated carbons (δ 82.3, 73.4) are tertiary, while the third (δ 74.1) belongs to a quaternary carbon. Three of the olefinic carbons (δ 136.7, 135.5, 130.0) are nonprotonated vinyls, two are singly protonated (δ 128.3, 125.3), and a sixth olefinic signal is of an exocyclic methylene carbon (δ 127.9). The upfield region of the ^{13}C -nmr spectrum contains one methine, six methylene carbon, and four methyl carbon signals.

The ^1H nmr spectrum (300 MHz, CDCl_3) corroborates the presence of four methyl groups (singlets at δ 1.96, 1.71, 1.59, 1.42) and several methylenes in the aliphatic region. A series of multiplets in the olefinic region substantiate the presence of several sites of unsaturation. Resonating at δ 5.70 and 6.45 were a set of doublets which were long-range-coupled ($J = 2.7$ Hz) to a proton resonating at δ 2.68, suggesting the presence of an exocyclic methylene. A heteronuclear chemical shift correlation with broad band decoupling [CSCMBB] (5,6) experiment correlated all proton resonances with their corresponding carbon resonances, including those in the aliphatic region. By use of a ^1H - ^1H correlation (COSY) (7,8) experiment coupled with a proton-proton double quantum (PDQ) (9–11) experiment, the



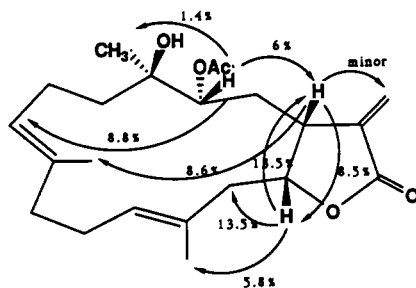


FIGURE 1. Structure of styelolide [1] demonstrated by nOe.

complete structure of **1** was assigned unequivocally, starting with the exocyclic methylene (δ 6.46 and 5.70, H-17) which exhibits a COSY response corresponding (allylic coupling) to the complex multiplet at δ 2.68. The single proton of this multiplet has responses to, and is therefore coupled to, the non-degenerate methylene protons (δ 2.20, 1.85, H-2) and a methine proton (δ 5.40, H-14). This latter proton and its corresponding carbon's chemical shift (δ 73.4, C-14) indicate the location of the attachment of the oxygen of the γ -lactone. It is important to note that the COSY response correlating these two protons is weak and that the PDQ experiment (optimized for 7 Hz coupling) lacks a corresponding response, suggesting that the two protons are *cis* in nature. A shift reagent study with $\text{Eu}(\text{fod})_3$ and an nOe study also support this argument.

The proton at position 14 (δ 5.40) also exhibits strong responses to the methylene protons at position 13 (δ 2.10 and 2.51), and the connectivity stopped there, thus indicating that C-12 (δ 135.5) must be a quaternary carbon. On the basis of biogenic considerations, a methyl (δ 1.71) was fixed at the sp^2 C-12. The chemical shift of this methyl (Me-20) would then be consistent with the literature. The methyl at δ 1.71 was allylically coupled (COSY) to the vinylic proton at δ 5.30 (H-11). Carrying the COSY and PDQ responses two steps further, we can then assign the resonances at δ 2.30 and 2.15 to H-10 and the resonance at δ 1.90 to H-9.

We have already established the connectivity between the protons of C-1 and those of the methylene protons of C-2. The later protons exhibit responses in both the COSY and PDQ experiments to the proton (dd, δ 3.94) on C-3. The chemical shift of this carbon (δ 82.3) also supports the location of the acetate group. Due to the lack of additional correlating responses, C-4 must be that of a quaternary carbon, and thus a methyl group could be fixed at C-4. This assignment is also supported by biogenic considerations. The ^1H nmr of **1**, in $\text{DMSO}-d_6$, showed an exchangeable singlet at δ 4.70, indicating that the hydroxyl group is present on a nonprotonated carbon. This assignment is also supported by the APT spectrum and the non-reactivity of **1** towards acetylation (pyridine/ Ac_2O). The placement of the hydroxyl group at position 4 is in accordance with chemical shift arguments, because the methyl protons lie at δ 1.41 (^1H -nmr spectrum) and the quaternary carbon resonates at δ 74.1 ppm in the ^{13}C -nmr spectrum. The shift reagent study substantiated this prediction.

The last vinyl proton at position 7 (δ 5.07) provides both COSY and PDQ responses connecting it to H-6 (δ 1.75, 2.35), which in turn correlates to the final methylene protons at position 5 (δ 1.59). Allylic coupling is also observed linking the vinyl proton to the methyl group resonating at δ 1.59. All protonated carbons from positions 5 through 11 are in accordance with known chemical shifts of similar cembranes (12–14). The carbon resonances are assigned by

the application of the CSCMBB experiment, and the three quaternary vinyl carbons are assigned by chemical shift arguments.

The relative stereochemistries of the four chiral carbons are based on coupling information, COSY and PDQ responses, a shift reagent, and nOe studies. The two protons at position 1 and 14 are cisoid to each other and to H-3. A minimal nOe of the methyl group at position 4 in relation to H-3 suggests a transoid relationship in this partially rigid portion of the molecule.

EXPERIMENTAL

S. plicata (5 kg wet wt) was collected in Panacea, Florida (Gulf Coast Specimen Company) from shallow water in December 1983 and stored in iPrOH. The tunicate was blended in iPrOH and filtered, and the solvent was evaporated to dryness. Solvent-solvent partition of the extract between hexane and 30% aqueous MeOH afforded 15 g of a dark brown oil from the hexane portion. This oil was chromatographed on a Si gel column (Baker, 60–200 mesh) using a solvent step gradient system from 50% hexane/50% CH₂Cl₂ to 100% CH₂Cl₂ in 5% increments. Repeated chromatography of the fraction from hexane-CH₂Cl₂ (10:90) on Si gel followed by chromatography using RP-18 (10% H₂O/90% MeOH) afforded 41 mg of styelolide and impurities as a light yellow oil. Final purification of styelolide was accomplished on a Waters hplc system equipped with a radial compression module (RCM 100) containing an RP-18 cartridge. An isocratic solvent separation [MeOH-H₂O (90:10)] was employed to yield 17 mg of a clear oil.

All nmr experiments were carried out on a Nicolet NT-300 wide bore spectrometer operating at 300.068 and 75.549 MHz for ¹H and ¹³C observations, respectively. The instrument was equipped with a Model 293-C pulse programmer and a 5 mm ¹H/¹³C dual tuned probe. Eims (70 eV) *m/z* [M - H₂O]⁺ 356, 321, 314, 263, 177, 109; ir ν max (CHCl₃) 3560, 1760, 1730, 1665 cm⁻¹; ¹H nmr (CDCl₃) δ 6.46 (1H, d, *J* = 2.4 Hz, H-17), 5.70 (1H, d, *J* = 2.2 Hz, H-17), 5.38 (1H, ddd, *J* = 8.5, 5.3, 2.8 Hz, H-14), 5.26 (1H, bd m, H-11), 5.07 (1H, bd t, *J* = 7.5 Hz, H-7), 3.94 (1H, bd d, *J* = 11.0 Hz, H-3), 2.68 (1H, m, H-1), 1.95 (3H, s, H-22), 1.71

(3H, s, H-20), 1.59 (3H, s, H-19), 1.41 ppm (3H, s, H-18); ¹³C nmr (CDCl₃) δ 170.6 (C-16), 167.3 (C-21), 136.7 (C-15), 135.5 (C-12), 130.0 (C-8), 128.3 (C-11), 127.8 (C-17), 125.2 (C-7), 82.3 (C-3), 74.1 (C-4), 73.4 (C-14), 41.6 (C-13), 39.9 (C-5), 38.6 (C-9), 37.4 (C-1), 24.8 (C-22), 23.1 (C-6), 22.1 (C-2), 20.2 (C-18), 14.8 (C-20), 14.5 (C-19).

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