Volvatellin, Caulerpenyne-Related Product from the Sacoglossan *Volvatella* sp.

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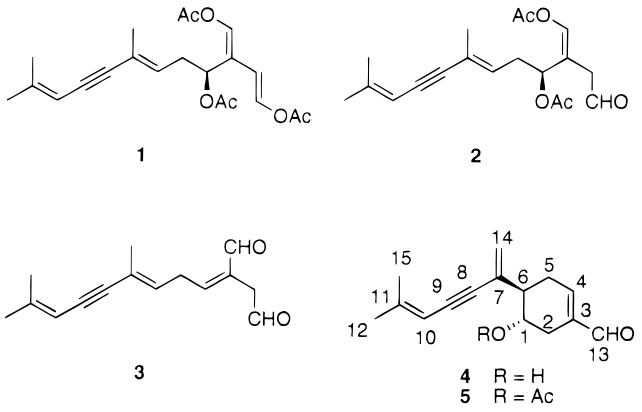
Volvatellin (**4**) is a highly unstable terpene isolated from the extracts of the Indian opisthobranch mollusk *Volvatella* sp. The structure and the relative stereochemistry of **4** were determined by NMR methods. The paper also describes a hypothetical biosynthesis of **4** starting from the alga-derived caulerpenyne.

Mediterranean sacoglossans (Mollusca, Gastropoda) have been extensively studied with respect to chemical defense[1−3] and evolution.[4] Such mollusks seem to be specialists in their feeding habits.[3,4] In particular, all Mediterranean shelled forms live in close association with the green alga *Caulerpa prolifera*.[3] The plant contains the toxic metabolite caulerpenyne (**1**), which acts to defend the alga from grazing.[5,6] However, the sacoglossans seem to be able to feed upon the alga and to use its chemicals for their own defense by modifying caulerpenyne (**1**) to the more active oxytoxin-1 (**2**) and oxytoxin-2 (**3**).[1−3] Recently, Pietra and co-workers have isolated a plethora of caulerpenyne-related compounds in the organic extracts of *Caulerpa taxifolia* from the Mediterranean Sea.[7,8] Although most of these products seem to be due to spontaneous, non enzymatic transformations of **1**, this finding raises the question of whether the sacoglossan metabolites may be produced by the animals by transforming **1**, or whether the opisthobranchs may simply accumulate minor algal compounds. Against this latter hypothesis is the fact that the Mediterranean *Oxynoe olivacea* and *Lobiger serradifalci* contain such large amounts of oxytoxins that the crude lipid extract from the autotomizable parts of their body was revealed to be pure **2** by ^{1}H NMR analysis.[1]

In support of the ability of sacoglossan mollusks to biomodify dietary metabolites, we report herein the new caulerpenyne derivative **4** isolated from the Indo-Pacific mollusk *Volvatella* sp. Compound **4**, which we have named volvatellin, gives us the chance to discuss the formal biosynthesis of the sacoglossan chemicals again.

The oxynoacean *Volvatella* Pease 1860 is one of the most primitive members of the gastropod order Sacoglossa. The genus is featured by a conspicuous shell and by a posterior siphon from which white defensive secretions are released when the animal is alarmed.[9] The frozen biological samples of *Volvatella* sp. and their secretions were collected on *Caulerpa* sp. off Mandapam (India) in June 1998. The population of mollusks consisted of 10 larger animals (ca. 10 mm in length) that carried on their shells, as a whole, 23 smaller individuals (ca. 3 mm in length). The frozen biological samples were separately extracted, and the diethyl ether-soluble fractions were analyzed by TLC. Algal and animal extracts contained caulerpenyne (**1**), but only the mollusks and their mucus showed also a more polar component that was later characterized as volvatellin (**4**). In fact, sequential SiO_2 column chromatography and HPLC



allowed us to purify **4** (0.8 mg) from the extracts of the large-sized specimens. The ^{1}H NMR spectrum of volvatellin (**4**) was featured by a prominent aldehyde signal at δ 9.47 (H-13) that weakly coupled the vinyl proton at δ 6.76 (H-4). The olefin region also contained resonances for an exomethylene group (H_2-14, δ 5.52 and 5.42) partially overlapped with the signal at δ 5.38 (H-10) (see Table 1). The ^{1}H NMR spectrum was completed by a deshielded methine signal at δ 3.92 (H-1), coupled both with the methylene hydrogens at δ 2.87 and 2.10 (H_2-2) and with the methine proton at δ 2.45 (H-6). This last signal showed further correlation with the multiplet centered at δ 2.56 (H_2-5). Considering the structure of **1**, the above-reported NMR data were in good agreement with the formula of volvatellin (**4**).

The depicted relative stereochemistry of the cyclohexene ring was established on the basis of the multiplicities of H-1 and H-6 (Table 1). In fact, both these protons showed two large (J a. 10 Hz) and one small (J ca. 5 Hz) coupling constants. Selective decoupling of the signal at δ 2.87 (H-2eq, dd, J = 17.1 and 5.5 Hz) simplified the hydroxyl deshielded proton into a triplet with an apparent J of 9.9 Hz. This clearly proved that H-1 was axial and coupled with other two axial hydrogens (H-2ax and H-6), thus suggesting the trans diequatorial relationship of the hydroxy group at C-1 with the alkyl chain at C-6.

Although the cyclic structure of volvatellin (**4**) may derive from caulerpenyne (Scheme 1), its molecular elucidation deserved far more detail. Because **4** decomposed into unidentified products, a second preparation of the terpenoid was carried out starting from the acetone extract of the small-sized specimens of *Volvatella*. However, to prevent any decomposition of the product, **4** was purified as reported above and then acetylated in dry pyridine to give

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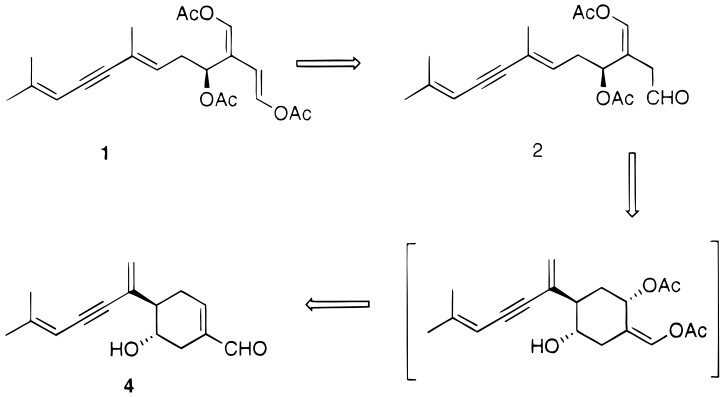
Table 1. NMR Data ($CDCl_3$) and $^1H-^{13}C$ Long-Range ($J_{^1H-^{13}C}$ = 4 or 9 Hz) Correlations of Volvatellin (**4**) and Acetyl Volvatellin (**5**)

	volvatellin (**4**)	acetyl volvatellin (**5**)		
position	$\delta_{^1H}$, m	$\delta_{^1H}$, m	$\delta_{^1H}$, m	HMBC (9 and 4 Hz)
1	3.92, dt (J = 10.0, 10.0, 5.3 Hz)	5.13, dt (J = 9.8, 9.8, 5.8 Hz)	69.8 (d)	H-6, H-2_{eq}
2	2.87, brdd (J = 17.1, 5.5 Hz)	2.88, brdd (J = 17.8, 5.8 Hz)	31.0 (t)	
	2.10, m	2.09, m		
3			138.6 (s)	H-13, H-2_{eq}
4	6.76, bd	6.79, brs	148.0 (d)	
5		2.70, m	26.9 (t)	H-13
		2.62, m		
6	2.45, brddd (J = 10.3, 10.3, 5.5 Hz)	2.69, m	45.4 (d)	H-14a
7			131.6 (s)	H-6
8			88.3 (s)	H_3-12
9			89.1 (s)	H_3-15, H-6
10	5.38, br s	5.35, br s	104.9 (d)	H_3-12, H_3-15
11			149.5 (q)	H_3-12, H_3-15
12	1.83, s	1.89, s	21.2 (q)	H_3-15
13	9.47, s	9.46, s	192.6 (d)	
14	5.52, d (J = 1.8 Hz)	5.43, d (J = 1.4 Hz)	122.0 (t)	H-6
	5.42, d (J = 1.8 Hz)	5.31, d (J = 1.4 Hz)		
15	1.81, s	1.81, s	24.9 (q)	H_3-12
Ac		2.03, s	21.0 (s)	
Ac			179.8 (s)	

Scheme 1. Formal Biotransformation of Caulerpenyne (**1**) into Volvatellin (**4**)



0.4 mg of the acetyl derivative **5**. 1D- and 2D NMR experiments proved the suggested structure to be correct and allowed the complete characterization of the compound (Table 1). In particular any other structure was ruled out by HMBC experiments (J = 4 and 10 Hz) that revealed correlations both of the methyl groups at δ 1.89 (CH_3-12) and δ 1.81 (CH_3-15) with C-10 (δ 104.9) and of the exomethylene protons (δ 5.43 and 5.31, H_2-14) with C-8 (δ 88.3) and C-6 (δ 45.4).

Finally, GC−MS analysis of the white secretion released by the molested animals revealed the significant occurrence of **4**. Analogously to the oxynocean mollusks from the Mediterranean Sea that have been studied, *Volvatella* sequesters and transforms algal metabolites. In our opinion, the finding of volvatellin (**4**) gives conclusive evidence of the ability of this group of shelled sacoglossans to process caulerpenyne. The cyclic, optically active structure of volvatellin (**4**, $[\alpha]^{20}_D$ −88.3, Et_2O) should derive from enzymatic transformation of **1**. In fact, in a biosynthetic pathway similar to that proposed for **2** and **3**, one may expect the conversion of caulerpenyne (**1**) into volvatellin (**4**) via a two-step process, such as illustrated in Scheme 1.

In conclusion, our data proved that **4** is present both in the mantle and in the white mucus expelled by the Indian opisthobranchs. This is in agreement with anatomical studies that suggested the storage of the defensive allomones in a mantle cavity connected to the siphonal spout at the rear of the shell.[10] Although we could not prove whether volvatellin (**4**) is a real defensive allomone or simply a way of detoxifying or storing other, more active (and unstable) products, the circumstantial evidence strongly supports the former hypothesis.

Experimental Section

General Experimental Procedures. 1D and 2D NMR spectra were recorded on a Bruker AMX-500. The $CHCl_3$ resonances at δ 7.26 and 77.0 were used as internal references. MS were obtained on a Kratos MS 50 spectrometer operating at 70 eV. IR data were recorded by BIO-RAD FTS-7 FT/IR spectrophotometer. Optical rotations were determined with a JASCO DIP-370 polarimeter. HPLC was performed using a Waters liquid chromatography apparatus equipped with two 510 pump units and a JASCO Uvidec 100 III spectrophotometer.

Collection, Extraction, and Purification. The nudibranchs (10 individuals of 10-mm length and 23 individuals of 3-mm length) and the alga *Caulerpa* sp. were collected off Mandapam (India) in June 1998. Voucher specimens are kept at ICMIB (MAN4 and MAN4a). The frozen animals were separately extracted with Me_2CO. After removing the volatile solvent, the residues were diluted with fresh water and separately partitioned by Et_2O. The organic layers were dried over Na_2SO_4, filtered on paper, and evaporated to give 64 mg from the large-sized mollusks, 19 mg from the small-sized mollusks, and 162 mg from the alga. After TLC comparison, the extracts were fractionated on Si gel to give **1** and **4** from the mollusk, and only **1** (2.2 mg) from the alga. Fractions containing **4** were further purified by reversed-phase HPLC column (analytical Spherisorb ODS-2) with a linear gradient from 50% to 90% of MeOH in H_2O (detector UV 254 nm) to

afford pure **4** (0.8 mg and 0.6 mg from large and small mollusks, respectively). Compound **4** from the smaller specimens of *Volvatella* was treated with Ac_2O (125 μL) in dry pyridine (400 μL). The organic solvent was removed under reduced pressure, and the reaction mixture was purified on a Si gel column to give **5** (0.4 mg).

Volvatellin (**4**): pale yellow oil (0.8 mg), $[\alpha]_D$ − 88.3° (*c* 0.04, Et_2O); UV λ_{max} (EtOH) 277 (8700), 263 (9430), 229 (15 630), 214 (16 900) 204 (20 100); IR (film) ν_{max} 3444, 1684 cm^{-1}; NMR data, see Table 1; EIMS (*m*/*z*) 230 (15), 229 (5), 202 (100), 187 (60), 115 (40).

Acetyl volvatellin (**5**): pale yellow oil (0.4 mg), $[\alpha]_D$ − 57.2° (*c* 0.08, $CHCl_3$); IR 1745, 1684 cm^{-1}.

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