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Climacostol, a defense toxin of the heterotrich ciliate *Climacostomum virens* against predators

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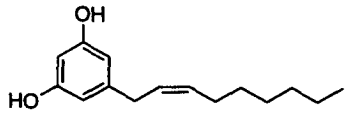
Abstract

A toxic substance (climacostol) of the protozoan ciliate *Climacostomum virens* against the predatory ciliate *Dileptus margaritifer* was established as 1,3-dihydoxy-5-[(*Z*)-2′-nonenyl]benzene. The structure was rigorously confirmed by the total synthesis.

Keywords: biologically active compounds; toxins; phenols; cobalt; cobalt compounds.

The heterotrich ciliates, *Stentor coeruleus* and *Blepharisma japonicum*, have pigment granules which contain blue pigment stentorin[1] and red pigment blepharismin,[2] respectively. We recently reported that the pigment granules and the pigments act as a defense against the predatory ciliate, *Dileptus margaritifer*.[3] Although *Climacostomum virens*, another heterotrich ciliate, is colorless, it has cortical vesicles, which are morphologically similar to the pigment granules in the red species of *Blepharisma*. The retreating behavior of *D. margaritifer* observed when it attacks *C. virens* suggests that *C. virens* also has the defense toxin against the predator. Indeed, we found a potent toxin against *D. margaritifer* in 70% ethanol extract of *C. virens*. In this paper we describe the isolation and structural determination of a new toxic substance, climacostol (**1**) of *C. virens*, a lethal toxin against the predator.



Climacostol (**1**)

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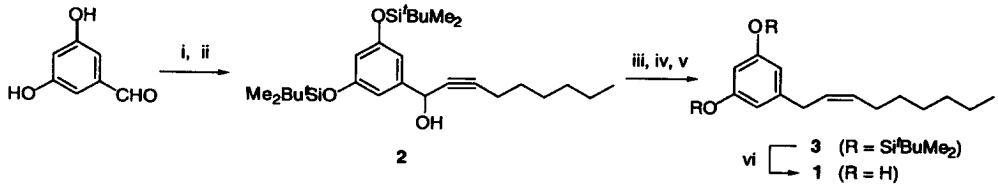
1. The isolation and structural determination of climacostol

The whole cells of *C. virens* were dipped in aqueous 70% EtOH. After removal of the cells by filtration and concentration of the solvent, the residue was partitioned between ethyl acetate and water. From the organic layer, an active fraction based on lethal toxicity against the predator *D. margaritifer* was obtained by repeated chromatography on silica gel eluted with MeOH:CH_2Cl_2, 7:93 (R_f value=0.26) and AcOEt:hexane, 1:1 (R_f value=0.5). The fraction contained a new compound climacostol (**1**) (LD_{50} 1.8 μg/ml),[4] whose molecular formula was estimated to be $C_{15}H_{22}O_2$ by HRMS and ^1H and ^{13}C NMR spectroscopy.[5] The presence of two phenolic OH protons and the close resemblance of the chemical shifts of the aromatic protons at δ 6.25 (2H, d, *J*=2.2 Hz, H-4, 6) and 6.18 (1H, t, *J*=2.2 Hz, H-2) and the carbons at δ 156.7 (2C, C-1, 3), 144.4 (C-5), 108.1 (2C, C-4, 6) and 100.4 (C-2) of **1** to that of olivetol (1,3-dihydoxy-5-pentylbenzene) suggested that **1** is a derivative of 5-alkenyl resorcinol. The ^1H–^1H COSY, HMBC, and HMQC spectra of **1** indicated the presence of Ar-CH_2-CH=CH-CH_2 (the olefinic protons at 5.51 (2H, m, H-2′, 3′) couple with protons at 3.28 (2H, d, *J*=6.1 Hz, H-1′) and 2.11 (2H, m, H-4′)), from which is deduced the presence of the 2′-nonenyl group as the side chain attached to resorcinol at the 5-position. The *Z*-configuration of Δ2′,3′-olefin was determined by the observation of NOE between the 1′- and 4′-methylene protons. Thus, the structure of **1** was established to be 1,3-dihydoxy-5-[(*Z*)-2′-nonenyl]benzene.

2. The synthesis of climacostol

The structure and biological activity of climacostol were confirmed by the synthesis of **1** as depicted in Scheme 1.[6] Since the alkynylation protocol[7] of 1-octyne to 3,5-dialkoxybenzylhalide using copper(I) halide as catalyst was less effective, the addition reaction of 1-octynyllithium to 3,5-dialkoxylbenzaldehyde was carried out. The bis-phenolic OH groups of commercially available 3,5-dihydroxybenzaldehyde were protected by the *tert*-butyldimethylsilyl groups since the more conventional methoxy groups were found not to be removed at the final stage. The acetylenic carbons of **2** were protected by complexing with biscobalt octacarbonyl, since the reductive removal of the hydroxyl group of **2** and its olefin derivative was unsuccessful. Reduction of the OH group at C1′ of the biscobalt complex of **2** with triethylsilane and $BF_3 \cdot OEt_2$ followed by reductive removal of biscobalt carbonyl under Isobe's condition[8] afforded *Z*-olefin **3**.[9] Deprotection of the silyl group of **3** by HF·pyridine in THF gave **1** with spectra identical to those of the natural product. The toxic activity of synthetic **1** against *D. margaritifer* was as high as that of the natural product.



Scheme 1. *Reagents and conditions*: (i) TBDMSCl, imidazole, DMF, 25°C, 87%; (ii) 1-octyne/*n*-BuLi, THF, −78→25°C, 91%; (iii) $Co_2(CO)_8$, Et_2O, −20→25°C, 64%; (iv) Et_3SiH, $BF_3 \cdot OEt_2$, CH_2Cl_2, reflux, 30 min, 89%; (v) *n*-Bu_3SnH, 65°C, 3 h, 93%; (vi) HF·pyridine, THF, 0→25°C, 2 h, 86%

The structure, synthesis and biological activity of resorcinolic lipids were recently reviewed in detail.[10] More than 100 of the 5-alkyl and 5-alkenylresorcinol homologues have been found mainly from a variety of higher plants, including those in the Proteaceae, Anacardiaceae, Ginkogoaceaen and

Graminae families, but they very seldom occur in animal organisms. Resorcinolic lipids are reported to show significant biological activities,[10] including antibacterial, antiparasitic, and cytotoxic activity, as a growth regulator, inhibition of DNA and RNA synthesis,[11] inhibitory effect on enzymes,[12] interaction with biological membranes, and as a modulator of lipid oxidation. The biosynthesis of climacostol is expected to be oriented from C_{16}-polyketide followed by cyclization and decarboxylation. Stentorin is also likely to be generated from C_{16}-polyketide accompanied with the addition of C_1 resulting in 2,4,5,7-tetrahydroxy-3-isopropylanthrone, and followed by dimerization of the anthrone. We expect a close relationship between climacostol and stentorin in respect to the biogenetic pathway and toxic activity against predators despite the difference in structures. The molecular mechanisms of the toxicity of climacostol against predators are under investigation.

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4. LD_{50} was determined by the procedure described in Ref. 3(b).
5. Spectrum data for climacostol (**1**): HRMS *m/z* calcd. for $C_{15}H_{22}O_2$ (M^+): 234.1620; found: 234.1630; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.25 (2H, d, *J*=2.2 Hz, H-4, 6), 6.18 (1H, t, *J*=2.2 Hz, H-2), 5.51 (2H, m, H-2′, 3′), 4.67 (2H, s, OH), 3.28 (2H, d, *J*=6.1 Hz, H-1′), 2.11 (2H, m, H-4′), 1.35–1.42 (2H, m, H-5′), 1.25–1.35 (6H, m, H-6′, 7′, 8′), 0.89 (3H, t, *J*=6.9 Hz, H-9′); ^{13}C NMR ($CDCl_3$) δ 156.7 (2C, C-1, 3), 144.4 (C-5), 131.4 (C-2′), 127.3 (C-3′), 108.1 (2C, C-4, 6), 100.4 (C-2), 33.2 (C-1′), 31.7 (C-7′), 29.6 (C-6′), 29.0 (C-5′), 27.2 (C-4′), 22.6 (C-8′), 14.1 (C-9′).
6. For the synthesis of 5-alkylresorcinols, see: Alonso, E.; Ramon, D. J.; Yus, M. *J. Org Chem.* **1997**, *62*, 417–421, and references cited therein.
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9. All new compounds gave spectroscopic data in agreement with the assigned structures.
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