Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron 60 (2004) 7041–7048
Tetrahedron

Climacostol, a defense toxin of *Climacostomum virens* (protozoa, ciliata), and its congeners

Miyuki Eiraku Masaki,[a] Shouji Hiro,[a] Yoshinosuke Usuki,[a] Terue Harumoto,[b] Masayo Noda Terazima,[b] Federico Buonanno,[c] Akio Miyake[c] and Hideo Iio[a,*]

[a]*Department of Material Science, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan*
[b]*Graduate School, Nara Women's University, Nara 630-8506, Japan*
[c]*Department of Molecular, Cellular and Animal Biology, University of Camerino, Camerino (MC) 62032, Italy*

Received 22 July 2003; revised 17 September 2003; accepted 17 September 2003

Available online 19 June 2004

Abstract—Climacostol (**1**), a defense toxin of the heterotrich ciliate *Climacostomum virens* was established as 5-(*Z*)-non-2-enyl-benzene-1,3-diol. The structure was rigorously confirmed by the total synthesis. The two congeners of climacostol contained in this ciliate were determined as 5-(*Z*,*Z*)-undeca-2,5-dienyl-benzene-1,3-diol (**2**) and 5-(*Z*,*Z*,*Z*)-undeca-2,5,8-trienyl-benzene-1,3-diol (**3**).

1. Introduction

The heterotrich ciliates *Stentor coeruleus* and *Blepharisma japonicum*, respectively, have a blue pigment stentorin[1] and red pigments blepharismins[2] in their extrusive organelles (pigment granules).[3] These pigments have been studied as photoreceptors,[4] but recently we showed that they also have the function of chemical defense against predators.[5] In another heterotrich ciliate *Climacostomum virens*, we found a defense toxin contained in its colorless extrusive organelles (cortical granules),[6] identified it as 5-(*Z*)-non-2-enyl-benzene-1,3-diol, and named it climacostol.[7] We also found that this ciliate has two congeners of climacostol. In this paper, we describe isolation, characterization and synthesis of climacostol and the structural elucidation of its congeners in detail.

2. Result and discussion

2.1. Isolation of climacostol and its congeners

Whole cells (0.8 mL) of *Climacostomum virens* were dipped in aqueous 75% EtOH (39 mL). After removal of the cells by filtration and concentration of the filtrate, the residue was partitioned between dichloromethane and water. From the organic layer, a biologically active fraction which is highly toxic against the raptorial ciliate *Dileptus margaritifer* was obtained by preparative TLC on silica gel developed with MeOH/CH_2Cl_2 7:93. The biologically active fraction contained climacostol and compounds **A** and **B**. The mixture were further purified by preparative TLC impregnated with 15% silver nitrate which was prepared in CH_3CN[8] (developing solvent MeOH/CH_2Cl_2 1:9) to give three fractions **A** (R_f value=0.31), **B** (R_f value=0.17) and **C** (R_f value=0.05). The fraction **A** (1.0 mg), the fraction **B** (0.1 mg) and the fraction **C** (0.2 mg) consisted, respectively, of mostly pure climacostol (**1**), a 1:1 mixture of **1** and new compound **2**, and mostly pure new compound **3**. We have quitted further purification of the fraction B due to its scarcity. LD_{50} of **1** for *D. margaritifer* (D3-I) was 0.71 μg/ml.[6]

2.2. Structure of climacostol and its congeners

The molecular formula of climacostol (**1**) was estimated as $C_{15}H_{22}O_2$ by high-resolution mass spectroscopy (HRMS): *m/z* $[M]^+$ found 234.1630, calcd 234.1620. In the preliminary study, the ^1H and ^{13}C NMR spectra of a mixture of climacostol (**1**), **2** and **3** had been recorded on the machine for 500 MHz, and the result was published in the preliminary paper.[7] Herein, pure climacostol (**1**) was submitted to measure the ^1H and ^{13}C NMR spectra on the machine for 600 MHz. The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of two phenolic OH protons and the close resemblance of chemical shifts of the aromatic protons at δ 6.25 (2H, d, *J*=1.9 Hz, H-4, 6) and 6.18 (1H, t, *J*=1.9 Hz, H-2) and carbons at δ 156.8 (2C, C-1, 3), 144.4 (C-5), 108.0 (2C, C-4, 6) and 100.4 (C-2) of **1** to that of olivetol (5-pentyl-benzene-1,3-diol) suggested that **1** is a derivative of 5-alkenyl resorcinol. The $^1H-^1H$ COSY

Keywords: Biologically active compounds; Toxins; Phenols; Resorcinol; Cobalt compounds.
* Corresponding author. Tel.: +81-6-6605-2562; fax: +81-6-6605-3152; e-mail address: iio@sci.osaka-cu.ac.jp

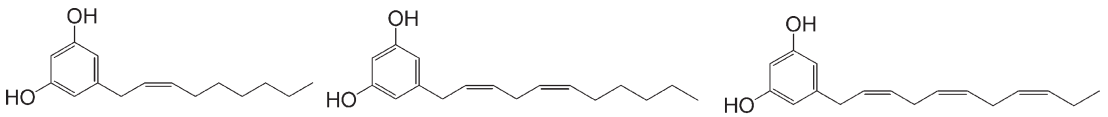
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.09.105

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data for climacostol (**1**) in $CDCl_3$

Position	δ_H	δ_C	COSY	HMBC	NOEsy
1,3	—	156.8 (2C)			
2	6.18 (1H, t, *J*=1.9 Hz)	100.4		1, 3, 4, 6	
4,6	6.25 (2H, d, *J*=1.9 Hz)	108.0 (2C)	1′	1, 2, 3, 1′	
5	—	144.4			
1′	3.28 (2H, d, *J*=6.1 Hz)	33.2	4, 6, 2′	4, 5, 6, 2′, 3′	4′
2′	5.50 (1H, m)	127.3	1′	1′, 4′	
3′	5.51 (1H, m)	131.5	4′	1′, 4′, 5′	
4′	2.11 (2H, q, *J*=6.9 Hz)	27.3	3′, 5′	2′, 3′, 5′	1′
5′	1.35–1.41 (2H, m)	29.7	4′	3′, 4′, 6′, 7′	
6′	1.25–1.34 (2H, m)	29.0		5′, 7′, 8′	
7′	1.25–1.34 (2H, m)	31.8		5′, 6′, 8′, 9′	
8′	1.25–1.34 (2H, m)	22.6	9′	6′, 7′, 9′	
9′	0.89 (3H, t, *J*=6.9 Hz)	14.1	8′	7′, 8′	
1,3 –OH	4.70 (2H, s)				

spectrum of **1** indicated that the presence of Ar–CH_2–CH=CH–CH_2. The olefinic protons at δ 5.50 (1H, m, H-2′) and 5.51 (1H, m, H-3′) were correlated with the protons at δ 3.28 (2H, d, *J*=6.1 Hz, H-1′) and 2.11 (2H, q, *J*=6.9 Hz, H-4′), which deduced the presence of a 2′-non-enyl group as the side chain attached to resorcinol at the 5-position. The side chain of **1**, in especially C-6′, 7′, 8′ was assigned by the HMQC and HMBC spectra (Table 1). The EI-MS/MS experiment was carried out in positive mode by choosing the $[M]^+$ ion (*m*/*z* 234) as the precursor ion. The fragment ion peaks of the EI-MS/MS spectrum were observed at *m*/*z* 219 $[M-CH_3]^+$, 205 $[M-C_2H_5]^+$, 191 $[M-C_3H_7]^+$, 177 $[M-C_4H_9]^+$, 163 $[M-C_5H_{11}]^+$, 149 $[M-C_6H_{13}]^+$, 124 $[M+H-CH=CHC_6H_{13}]^+$, which confirmed the presence of double bonds at $\Delta^{2,3}$ as shown in Figure 1. The *Z*-configuration of $\Delta^{2',3'}$-olefin was determined by the observation of NOE between the 1′- and 4′-methylene protons. Thus, the structure of a new toxic compound climacostol (**1**) was determined to be 5-(*Z*)-non-2-enyl-benzene-1,3-diol. Previously, the structure of 5-(*Z*)-heptadec-2-enyl-benzene-1,3-diol, a derivative of 5-(2′-alkenyl)-resorcinol, was reported as a possible dermatitis allergen from the latex of a mango.[9] The position of the olefin was assumed from the chemical shifts of ^{1}H NMR (CCl_4, 60 MHz) δ 5.33 (2H, br t, H-2′, 3′), 2.25–2.60 (2H, m, H-1′), 1.80–2.20 (2H, m, H-4′), and fragment peaks of MS. The incompatibility of the data to that of climacostol suggests the necessity of reinvestigating the structure of the compound from the mango. To our best knowledge, climacostol is the first example of 5-alkenylresorcinol having a double bond at the 2′-position.

Analysis of ^{1}H NMR for compound **2** was carried out in the 1:1 mixture of climacostol and **2**. The ^{1}H NMR spectra (Table 2) of **2** showed the presence of two phenolic OH protons, and the aromatic protons at δ 6.26 (2H, d, *J*=2.3 Hz, H-4, 6) and 6.18 (1H, t, *J*=2.3 Hz, H-2) suggested that **2** is also a derivative of 5-alkenyl resorcinol. The ^{1}H



1 (Climacotol) **2** **3**

Figure 1. Climacostol and its congeners from *Climacostomum virens*.

Table 2. ^{1}H NMR (600 MHz) data of **2** and **3** in $CDCl_3$

	2		**3**		
Position	δ_H	NOEsy	δ_H	COSY	NOEsy
2	6.18 (1H, t, *J*=2.3 Hz)		6.19 (1H, t, *J*=2.3 Hz)	4,6	
4,6	6.26 (2H, d, *J*=2.3 Hz)		6.25 (2H, d, *J*=2.3 Hz)	2	1′
1′	3.31 (2H, d, *J*=6.3 Hz)	4′	3.32 (2H, d, *J*=6.6 Hz)	2′	6,4′
2′	5.47–5.56 (1H, m)		5.51–5.55 (1H, m)	1′	
3′	5.47–5.56 (1H, m)		5.51–5.55 (1H, m)	4′	
4′	2.87 (2H, t, *J*=6.5 Hz)	1′, 7′	2.91 (2H, t, *J*=5.6 Hz)	3′, 5′	1′, 7′
5′	5.34–5.44 (1H, m)		5.38–5.42 (1H, m)	4′	
6′	5.34–5.44 (1H, m)		5.38–5.42 (1H, m)	7′	
7′	2.04–2.08 (2H, m)	4′	2.82 (2H, t, *J*=5.5 Hz)	6′, 8′	4′, 10′[a]
8′	ND		5.31–5.36 (1H, m)	7′	
9′	ND		5.38–5.42 (1H, m)	10′	
10′	ND		2.06–2.08 (2H, m)	9′, 11′	7′[a]
11′	ND		0.97 (3H, t, *J*=7.5 Hz)	10′	
1,3 –OH	4.67 (2H, s)		4.68 (2H, s)		

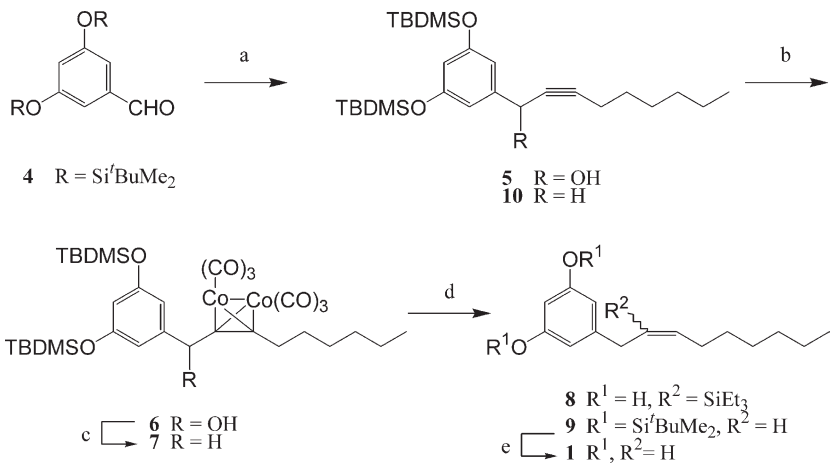
ND, not determined.
[a] Observed in benzene-d_6.

NMR spectrum also showed the presence of the olefinic protons at δ 5.47–5.56 (2H, m, H-2′, 3′), 5.34–5.44 (2H, m, H-5′, 6′), the benzylic methylene protons at δ 3.31 (2H, d, *J*=6.3 Hz, H-1′), the double-allylic methylene protons at δ 2.87 (2H, t, *J*=6.5 Hz, H-4′), and the allylic methylene protons at δ 2.04–2.08 (2H, m, H-7′), indicating that the presence of a dienyl group as the side chain attached to resorcinol. Although the carbon length of **2** could not be determined from the ^{1}H NMR spectrum, comparison of the LC/ESI-MS/MS spectrum (vide infra) with that of **3** allowed us to elucidate the side chain of **2**. The LC/ESI-MS spectrum showed *m/z* 259 $[M-H]^-$ ion peak, which suggested the carbon length of the side chain as C11. The LC/ESI-MS/MS experiment was carried out on negative ions by choosing the $[M-H]^-$ ion (*m/z* 259) as the precursor ion. The fragment ion peaks of the LC/ESI-MS/MS spectrum were observed at *m/z* 244 $[M-H-CH_3]^-$, 230 $[M-H-C_2H_5]^-$, 215 $[M-2H-C_3H_7]^-$, 201 $[M-2H-C_4H_9]^-$, 187 $[M-2H-C_5H_{11}]^-$, 161 $[M-2H-CH{=}CHC_5H_{11}]^-$, 147 $[M-2H-CH_2CH{=}CHC_5H_{11}]^-$, 122 $[M-H-CH{=}CHCH_2CH{=}CHC_5H_{11}]^-$, 108 $[M-H-CH_2CH{=}CHCH_2CH{=}CHC_5H_{11}]^-$, which confirmed the presence of double bonds at $\Delta^{2,3}$ and $\Delta^{5,6}$ as shown in Figure 1. The configuration of the double bonds was determined to be both *cis* by the observation of NOE's between the 1′- and 4′-methylene protons, and the 4′- and 7′-methylene protons. Thus, the structure of **2** was established as 5-(*Z*,*Z*)-undeca-2,5-dienyl-benzene-1,3-diol.

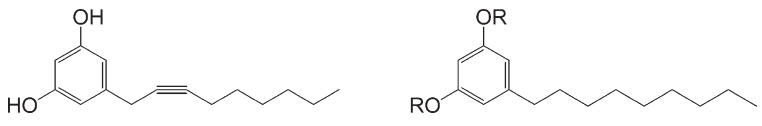
The molecular formula of compound **3** was estimated as $C_{17}H_{21}O_2$ by FAB-HRMS (*m/z* $[M-H]^-$ found 257.1528 calcd 257.1542). The ^{1}H NMR spectra (Table 2) showed the presence of two phenolic OH protons, and the aromatic protons at δ 6.25 (2H, d, *J*=2.3 Hz, H-4, 6) and 6.19 (1H, t, *J*=2.3 Hz, H-2), suggested that **3** is also a derivative of 5-alkenyl resorcinol. The ^{1}H–^{1}H COSY NMR spectrum of **3** showed that the olefinic proton at δ 5.51–5.55 (1H, m, H-2′), the olefinic protons at δ 5.51–5.55 (1H, m, H-3′) and 5.38–5.42 (1H, m, H-5′), the olefinic protons at δ 5.38–5.42 (1H, m, H-6′) and 5.31–5.36 (1H, m, H-8′), and the olefinic proton at δ 5.38–5.42 (1H, m, H-9′) were correlated, respectively, to the benzylic protons at δ 3.32 (2H, d, *J*=6.6 Hz, H-1′), the double-allylic methylene protons at δ 2.91 (2H, t, *J*=5.6 Hz, H-4′), the double-allylic methylene protons at δ 2.82 (2H, t, *J*=5.5 Hz, H-7′), and the allylic methylene protons at δ 2.06–2.08 (2H, m, H-10′), which suggested the side chain of **3** is the skipped triene at $\Delta^{2',3'}$, $\Delta^{5',6'}$ and $\Delta^{8',9'}$ with the carbon length of C11. The protons at δ 2.06–2.08 (2H, m, H-10′) and the triplet protons at δ 0.97 (3H, t, *J*=7.5 Hz, H-11′) showed the terminal ethyl function. Thus, the undeca-2′,5′,8′-trienyl group is added to resorcinol at the 5-position as the side chain. The *Z*-configuration of $\Delta^{2',3'}$-olefin, $\Delta^{5',6'}$-olefin and $\Delta^{8',9'}$-olefin were determined, respectively, by the observation of NOE between the 1′- and 4′-protons, 4′- and 7′-protons, and 7′- and 10′-protons. The proposed structure was further supported by LC/ESI-MS/MS spectrum. The LC/ESI-MS/MS experiment was carried out on negative ions by choosing the $[M-H]^-$ ion (*m/z* 257) as the precursor ion. The fragment peaks in the LC/ESI-MS/MS spectrum of **3** were observed at *m/z* 227 $[M-2H-C_2H_5]^-$, 202 $[M-H-CH{=}CHC_2H_5]^-$, 187 $[M-2H-CH_2-CH{=}CHC_2H_5]^-$, 161 $[M-2H-CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 147 $[M-2H-CH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 122 $[M-H-CH{=}CHCH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 108 $[M-H-CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$, which confirmed the position of three olefins at $\Delta^{2',3'}$, $\Delta^{5',6'}$ and $\Delta^{8',9'}$. Thus, the structure of **3** was determined to be 5-(*Z*,*Z*,*Z*)-undeca-2,5,8-trienyl-benzene-1,3-diol.

2.3. Synthesis of climacostol

The structure and biological activity of climacostol were confirmed by the synthesis of **1**.[10] The synthesis of climacostol (**1**) was achieved in six steps (Scheme 1). Phenolic hydroxyl groups of commercially available 3,5-dihydroxybenzaldehyde was protected with *tert*-butyldimethylsilyl groups, and reacted with 1-octyne using *n*-BuLi at −78 °C to give the acetylenic adduct **5** in 91% yield. Reductive removal of the hydroxyl group at C1′ of the adduct **5** was unsuccessful, because of migration of the unsaturated bond. The triple bond of **5** was protected by treatment with dicobalt octacarbonyl to afford the corresponding cobalt complex **6** in 64% yield. The hydroxyl group at the benzylic position of **6** was activated by complexation of triple bond with dicobalt octacarbonyl and the reduction of the hydroxyl group with triethylsilane in the presence of $BF_3{\cdot}OEt_2$[11] proceeded to afford **7** in 89% yield.



Scheme 1. Reagents and conditions. (a) 1-octyne, *n*-BuLi, THF, −78 °C, 91%. (b) $Co_2(CO)_8$, Et_2O, −20 °C→25 °C, 64%. (c) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 89%. (d) *n*-Bu_3SnH, benzene, 65 °C, 93%. (e) 70% HF·pyridine, THF, 86%.



11

12 R = Si^{t}BuMe$_2$
13 R = H

Figure 2. Synthetic analogues of climacostol.

In this reaction, a regio- and stereoselective hydrosilylation product was also formed as a minor side product, whose structure was confirmed by leading to **8**. The configuration of the olefin in **8** has not been determined. The hydrosilylation of an acetylene cobalt complex was also reported by Isobe et al.[12] The *Z*-olefin **9** was obtained by reductive decomplexation of the cobalt carbonyl under the Isobe's conditions[13] in 93%. Climacostol (**1**) was finally synthesized by deprotection of the silyl group of **9** in HF·pyridine. Synthetic climacostol (**1**) exhibited identical properties to those for the natural product. The toxic activity against *D. margaritifer* was as high as that of the natural product. Recently, Mori et al. reported a simpler synthesis of climacostol based on the Wittig reaction method starting from [3,5-bis-(*tert*-butyl-dimethyl-silanyloxy)-phenyl]-acetic acid methyl ester.[14]

The alkynyl and alkyl derivatives of climacostol were also synthesized from **7** and **9**, respectively, in order to do the biological evaluation. Thus, **7** was oxidized with I_2 to yield **10** in 90% yield, followed by deprotection of the silyl group by HF·pyridine to afford the alkynyl derivative **11** in quantitative yield. *Z*-olefin **9** was reduced with 10 wt% palladium-on-charcoal as a catalyst to give **12** in 80% yield, and deprotection of the silyl group afforded the alkyl derivatives **13** in 83% yield (Fig. 2).

2.4. The biosynthesis and the biological activity of climacostol

We consider that there is a close relation between the biosynthesis of climacostol and that of stentorin, a toxic pigment from *Stentor coeruleus*, though the chemical structure of them is considerably different to each other. A hypothetical pathway for the biosynthesis of long-chain resorcinolic lipids[15] would be also true for the biosynthesis of climacostol and its congeners. Namely, climacostol (C_{15}) and its congeners (C_{17}) would be synthesized from the C_{16}- and C_{18}-polyketide, respectively, with the cyclization and the decarboxylation, as shown in Figure 3. Stentorin is also likely generated from C_{16}-polyketide accompanied by the addition reaction of C_1 to form isopropyl group resulting 2,4,5,7-tetrahydroxy-3-isopropylanthrone, and followed by the dimerization reaction of the anthrone.[2a,16] The finding of

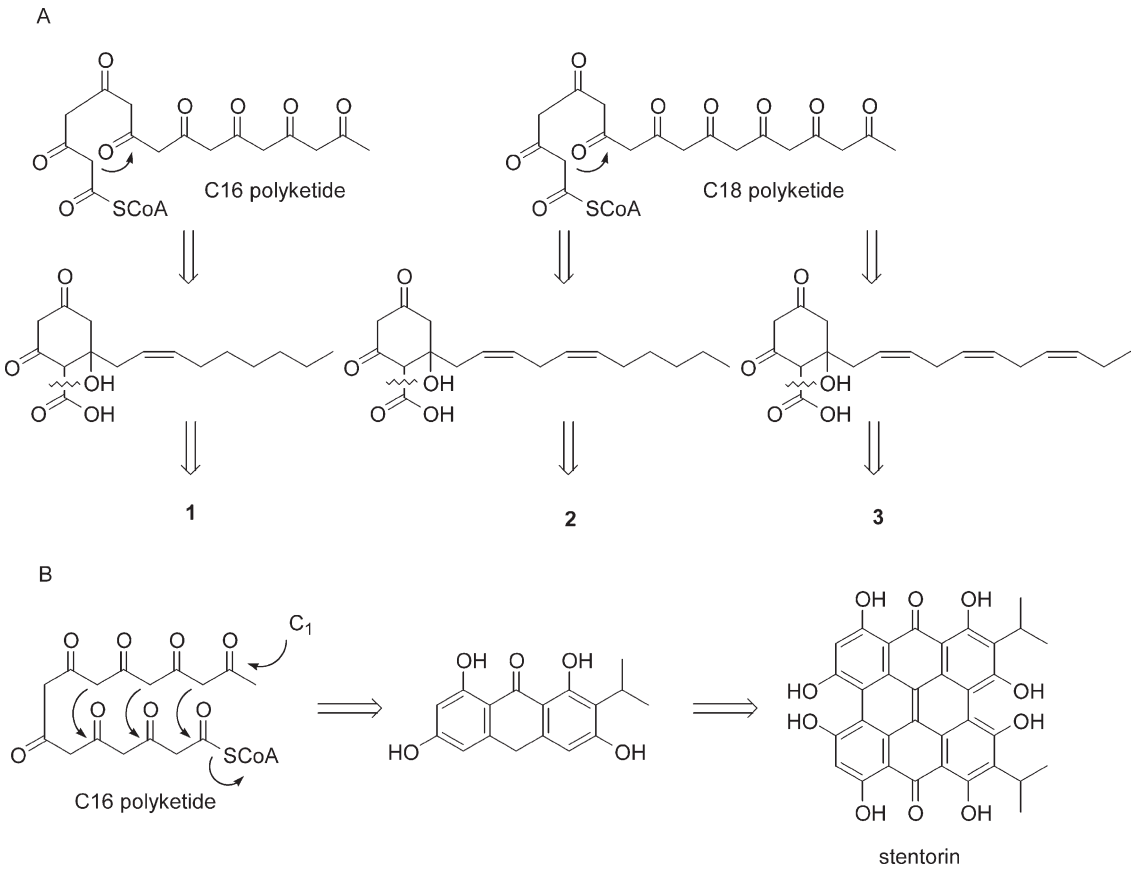


Figure 3. The putative biosynthesis of climacostol (**1**) and its congeners, and stentorin.

climacostol as defense toxin of *C. virens* is consisting with the suggestion that the primary function of stentorin and blepharismin is the defense against the predator rather than photoreception.[5b,17]

Cell–cell interaction by means of extrusomes (extrusive organelles in protist) in ciliates was reviewed by Miyake.[17] Secondary metabolites of ciliates were scarcely studied except the pigments mentioned above, keronopsin from *Pseudokeronopsis rubra*,[18] and euplotin,[19] raikovenal[20] and epoxyfocardin[21] from *Euplotes* species. The structure, synthesis and biological activity of resorcinolic lipids were recently reviewed in detail.[15,22] More than 100 of 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 rarely found in animal and microorganism. Resorcinolic lipids are reported to show significant biological activities,[15,22] including antibacterial, antiparasitic and cytotoxic activity as growth regulator, inhibition of DNA and RNA synthesis,[23] inhibitory effect on enzymes,[24] nematicidal activities,[25] interaction with biological membranes, and as modulator of lipid oxidation. In recent studies, climacostol was found to cleave DNA in the presence of $CuCl_2$,[26] and also climacostol specifically inhibits the respiratory chain complex I in mitochondria.[27]

Biological activities including lethal toxicity against the predatory ciliate *D. margaritiefer* of the congeners **2** and **3**, and the synthetic alkyl and alkynyl derivatives will be reported in due course.

3. Conclusion

A potent toxin against *D. margaritifer* was found in the 75% ethanol extract of *C. virens*. A new toxin climacostol of *C. virens*, a lethal toxin against the predator, was isolated and its structure was determined. The two congeners of climacostol were also isolated and their structures were determined. Furthermore, the synthesis of climacostol was carried out and the structure of the natural product was confirmed.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-600 for 600 MHz, Varian Unity-500 for 500 MHz, JEOL JNM-LA400 for 400 MHz or JEOL JNM-LA300 for 300 MHz. Chemical shifts (δ) are given in ppm relative to tetramethysilane (δ 0.00) or $CHCl_3$ (δ 7.26) for ^1H NMR and δ 77.0 for ^{13}C NMR as internal standard. Mass spectra were obtained on a JEOL JMS-700T. IR spectra were obtained on a JUSCO A-100. Reaction solvents were reagent grade. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl. Dichloromethane (CH_2Cl_2) was distilled from phosphorous oxide. Dimethyformamide (DMF) was distilled from calcium hydride. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates (Art5715 Kiesel gel $60F_{254}$ 0.25 mm), and preparative TLC was done on Merck silica gel plates (Art5744 Kiesel gel $60F_{254}$ 0.5 mm). Silica gel column chromatography was carried out on Daisogel IR-60 (63/210 μm).

4.2. Microbial material

Stock W-24 of *Climacostomum virens*, provided by Dr. Tavrovskaya (see Acknowledgement), was originally green due to symbiotic algae. By culturing in the dark, colorless clones (200–300 μm in length, 150–200 μm in width) were obtained. Stock D3-I of *D. margaritifer* (ca. 800 μm in length, ca. 60 μm in width) formerly *D. anser*, provided by Dr. Tavrovskaya, was used as a predator of *C. virens*. Both ciliates were grown on *Sathrophilus* sp., a small ciliate grown on the boiled lettuce medium, and concentrated by centrifugation, washed by and suspended in SMB-III,[28] a balanced salt solution (called SMB below).[6] Culture, handling of ciliates and experiments were performed at 24±1 °C.

4.3. Biological assay

Toxicity-test of climacostol: Ten cells of a ciliate were placed in 250 μL of SMB solution of climacostol of various concentrations, kept in a dark moist chamber, and the number of surviving cells were counted after 24 h. The LD_{50} concentration of climacostol for a ciliate was obtained based on the concentration–survival curve of the ciliate. At the late stage of this work, we noticed that the way to dilute the stock solution of climacostol (5 mg/mL ethanol solution) influences the result of toxicity measurement of climacostol. For example, if the ethanol solution was first diluted at 100, 10, and 5 μg/mL and then further diluted, measured LD_{50} concentrations for *D. margaritifer* (D3-I) were 1.8, 0.88, and 0.71 μg/mL, respectively.[6]

4.4. Extraction and isolation

Whole cells (0.8 mL) of *Climacostomum virens* were dipped in aqueous 75% EtOH (39 mL). After removal of the cells by filtration, the EtOH solution was concentrated under the reduced pressure. To the residue was added water and then extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was subjected to the preparative TLC (developed with MeOH/CH_2Cl_2, 7:93), and then eluted with MeOH/CH_2Cl_2, 1:4. The biologically active fraction was further purified by the preparative TLC impregnated with 15% silver nitrate which was prepared in CH_3CN.[8] The residue was dissolved in CH_2Cl_2, charged on the preparative TLC impregnated with 15% silver nitrate in CH_3CN. The plate was developed with MeOH/CH_2Cl_2, 1:9. Three fractions were observed by 2.5% $FeCl_3{\cdot}6H_2O$ in EtOH. Each of fractions was scraped off and eluted with MeOH/CH_2Cl_2, 1:4 to give climacostol (**1**) (1.0 mg, R_f value=0.31), a 1:1 mixture of **1** and **2** (0.1 mg, R_f value=0.17), and **3** (0.2 mg, R_f value=0.05).

4.4.1. Climacostol (1). ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; EI-MS *m/z* 234 $[M]^+$; EI-MS/MS *m/z* 219 $[M-CH_3]^+$, 205 $[M-C_2H_5]^+$, 191 $[M-C_3H_7]^+$, 177 $[M-C_4H_9]^+$, 163 $[M-C_5H_{11}]^+$, 149

$[M-C_6H_{13}]^+$, 124 $[M+H-CH{=}CHC_6H_{13}]^+$. HRMS (FAB^+) calcd for $C_{15}H_{22}O_2$ $[M]^+$ 234.1620, found 234.1630.

4.4.2. 5-(*Z*,*Z*)-Undeca-2,5-dienyl-benzene-1,3-diol (2). ^1H NMR ($CDCl_3$, 600 MHz), see Table 2; LC/ESI-MS (in negative mode) *m/z* 259 $[M-H]^-$; LC/ESI-MS/MS (in negative mode) *m/z* 244 $[M-H-CH_3]^-$, 230 $[M-H-C_2H_5]^-$, 215 $[M-2H-C_3H_7]^-$, 201 $[M-2H-C_4H_9]^-$, 187 $[M-2H-C_5H_{11}]^-$, 161 $[M-2H-CH{=}CHC_5H_{11}]^-$, 147 $[M-2H-CH_2CH{=}CHC_5H_{11}]^-$, 122 $[M-H-CH{=}CHCH_2CH{=}CHC_5H_{11}]^-$, 108 $[M-H-CH_2CH{=}CHCH_2CH{=}CHC_5H_{11}]^-$. HRMS ($EI^+$) calcd for $C_{17}H_{24}O_2$ $[M]^+$ 260.1776, found 260.1763.

4.4.3. 5-(*Z*,*Z*,*Z*)-Undeca-2,5,8-trienyl-benzene-1,3-diol (3). ^1H NMR ($CDCl_3$, 600 MHz), see Table 2; LC/ESI-MS (in negative mode) *m/z* 257 $[M-H]^-$; LC/ESI-MS/MS (in negative mode) *m/z* 227 $[M-2H-C_2H_5]^-$, 202 $[M-H-CH{=}CHC_2H_5]^-$, 187 $[M-2H-CH_2CH{=}CHC_2H_5]^-$, 161 $[M-2H-CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 147 $[M-2H-CH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 122 $[M-H-CH{=}CHCH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$, 108 $[M-H-CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CHC_2H_5]^-$. HRMS ($FAB^-$) calcd for $C_{17}H_{21}O_2$ $[M-H]^-$ 257.1542, found 257.1528.

4.5. Synthesis of climacostol and its derivatives

4.5.1. 1-[3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-phenyl]-non-2-yn-1-ol (5). 1-Octyne (720 mg, 6.5 mmol) was dissolved in THF (13 mL) and cooled to −78 °C under an argon atmosphere, and *n*-BuLi (1.6 M solution in hexane, 3.4 mL, 5.5 mmol) was added dropwise. After the mixture was stirred for 1.5 h at −78 °C, a solution of 3,5-bis-(*tert*-butyl-dimethyl-silanyloxy)-benzaldehyde (**4**) (2.0 g, 5.5 mmol) in THF (10 mL) was added at −78 °C. The reaction temperature was gradually raised to 25 °C and stirred overnight. To the reaction mixture was added saturated aqueous NH_4Cl and extracted with diethyl ether. The organic layer was washed with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to give **5** (2.36 g, 91%): ^1H NMR ($CDCl_3$, 400 MHz) δ 6.66 (2H, d, *J*=2.2 Hz, H-2, 6), 6.28 (1H, t, *J*=2.2 Hz, H-4), 5.31 (1H, br s, H-1′), 2.263 (1H, t, *J*=7.1 Hz, H-4′), 2.258 (1H, t, *J*=7.1 Hz, H-4′), 2.04 (1H, br s, 1′-OH), 1.50–1.57 (2H, m, H-5′), 1.36–1.43 (2H, m), 1.26–1.34 (4H, m), 0.98 (18H, s, Si–C$(CH_3)_3$), 0.88 (3H, H-9′), 0.20 (12H, s, Si–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6 (2C), 143.3, 111.70, 111.65 (2C), 87.4, 79.8, 64.6, 31.3, 28.6 (2C), 25.7 (6C), 22.5, 18.8, 18.2 (2C), 14.0, −4.4 (4C); IR (neat) 2980, 2955, 2925, 2880, 1605, 1465, 1405, 1380, 1350, 1270, 1175, 1040, 1015, 990, 950, 840, 790, 750, 690, 675 cm^{-1}; EI-MS *m/z* 476 $[M]^+$; HRMS (FAB^+) calcd for $C_{27}H_{48}O_3Si_2$ $[M]^+$ 476.3142, found 476.3157.

4.5.2. 1-[3,5-Bis-(*tert*-butyl-dimethyl-silanyloxy)-phenyl]-non-2-yn-1-ol cobalt complex (6). To a solution of **5** (2 g, 4.2 mmol) in diethyl ether (5 mL) was added a solution of $Co_2(CO)_8$ (2.1 g, 6.1 mmol) in diethyl ether (20 mL) at −20 °C under an argon atmosphere. The cooling bath was removed and the reaction mixture was stirred for 17.5 h at room temperature. The reaction mixture was washed with 1 M HCl. The organic layer was dried over Na_2SO_4, evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/AcOEt, 9:1) to give **6** (2.06 g, 64%): ^1H NMR ($CDCl_3$, 400 MHz) δ 6.54 (2H, d, *J*=1.7 Hz, H-2, 6), 6.23 (1H, t, *J*=1.7 Hz, H-4), 5.76 (1H, d, *J*=3.2 Hz, H-1′), 2.77 (1H, t, *J*=5.6 Hz, H-4′), 2.74 (1H, t, *J*=6.0 Hz, H-4′), 2.23 (1H, d, *J*=3.2 Hz, –OH), 1.58–1.69 (2H, m, H-5′), 1.42–1.49 (2H, m), 1.31–1.38 (4H, m), 0.98 (18H, s, Si–C$(CH_3)_3$), 0.91 (3H, t, *J*=7.1 Hz, H-9′), 0.185 (6H, s, Si–CH_3), 0.179 (6H, s, Si–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.9 (3C), 199.5 (3C), 156.8 (2C), 146.2, 111.6, 110.3 (2C), 101.5, 98.9, 73.9, 33.7, 31.9, 31.6, 29.3, 25.6 (6C), 22.6, 18.2 (2C), 14.0, −4.4 (4C); IR (nujol) 2975, 2950, 2880, 2120, 2080, 2050, 1603, 1470, 1390, 1350, 1265, 1180, 1040, 1020, 845, 790, 730 cm^{-1}; FAB-MS *m/z* 678 $[M-3CO]^+$ 650 $[M-4CO]^+$, 622 $[M-5CO]^+$, 594 $[M-6CO]^+$; HRMS (FAB^+) calcd for $C_{30}H_{48}O_6Si_2Co_2$ $[M-3CO]^+$ 678.1653, found 678.1664.

4.5.3. 1,3-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-non-2-ynyl-benzene cobalt complex (7). To a solution of **6** (1.1 g, 1.4 mmol) and Et_3SiH (0.22 mL, 1.4 mmol) in CH_2Cl_2 (14 mL) was added $BF_3{\cdot}OEt_2$ (0.16 mL, 1.4 mmol) dropwise under the argon atmosphere. After the mixture was refluxed for 30 min, the reaction mixture was added to saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure to give **7** (960 mg, 89%), and the hydrosilylation product as a minor product whose *tert*-butyldimethysilyl groups were removed by HF·pyridine to afford **8**; data for **7**: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.36 (2H, d, *J*=2.1 Hz, H-4,6), 6.22 (1H, t, *J*=2.1 Hz, H-2), 3.95 (2H, s, H-1′), 2.78–2.82 (2H, m, H-4′), 1.59–1.67 (2H, m, H-5′), 1.42–1.48 (2H, m), 1.31–1.37 (4H, m), 0.98 (18H, s, Si–C$(CH_3)_3$), 0.91 (3H, t, *J*=7.3 Hz, H-9′), 0.18 (12H, s, Si–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.9 (3C), 199.5 (3C), 156.75, 156.67, 146.1, 111.5, 110.3 (2C), 101.5, 98.9, 73.9, 33.9, 31.9, 31.6, 29.3, 25.6 (6C), 22.6, 18.1 (2C), 14.0, −4.5 (4C); IR (nujol) 2975, 2950, 2880, 2120, 2075, 2050, 1603, 1480, 1470, 1390, 1350, 1265, 1180, 1040, 1020, 840, 790 cm^{-1}; FAB-MS *m/z* 662 $[M-3CO]^+$, 635 $[M-3CO+H]^+$, 606 $[M-5CO]^+$, 461 $[M-Co_2(CO)_6+H]^+$; HRMS (FAB^+) calcd for $C_{30}H_{48}O_5Si_2Co_2$ $[M-3CO]^+$ 662.1704, found 662.1714; data for **8**: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.20 (2H, d, *J*=1.7 Hz, H-4,6), 6.15 (1H, t, *J*=1.7 Hz, H-2), 5.92 (2H, t, *J*=6.8 Hz, H-3′), 4.66 (2H, s, OH), 3.37 (2H, s, H-1′), 2.14 (2H, q, *J*=6.8 Hz, H-4′), 1.37–1.45 (2H, m, H-5′), 1.20–1.30 (6H, m, H-6′, 7′, 8′), 0.87 (3H, t, *J*=7.1 Hz, H-9′), 0.83 (9H, t, *J*=7.9 Hz, Si–CH_2CH_3), 0.46 (6H, q, *J*=7.9 Hz, Si–CH_2CH_3).

4.5.4. 1,3-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-non-2-enyl-benzene (9). To a solution of **7** (130 mg, 0.17 mmol) in benzene (1.7 mL) was added *n*-Bu_3SnH (0.09 mL, 0.34 mmol). After the reaction mixture was stirred for 3 h at 65 °C, the solvent was evaporated and then the residue was purified by silica gel column chromatography with *n*-hexane to give **9** (73 mg, 93%): ^1H NMR ($CDCl_3$, 400 MHz) δ 6.30 (2H, d, *J*=2.2 Hz, H-4, 6), 6.17 (1H, t, *J*=2.2 Hz, H-2), 5.50 (2H, t, *J*=4.8 Hz, H-2′, 3′), 3.27 (2H,

d, J=4.8 Hz, H-1′), 2.12 (2H, m, H-4′), 1.42–1.52 (2H, m, H-5′), 1.21–1.41 (6H, m, H-6′, 7′, 8′), 0.98 (18H, s, Si–C(CH$_3$)$_3$), 0.90 (3H, m, H-9′), 0.18 (12H, s, Si–CH$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 156.4 (2C), 143.2, 131.0, 127.8, 113.5 (2C), 109.5, 33.4, 31.8, 29.7, 29.0, 27.3, 25.7 (6C), 22.7, 18.2 (2C), 14.1, −4.5 (4C). IR (neat) 2950, 2925, 2850, 1590, 1450, 1335, 1250, 1160, 1020, 1000, 830, 775 cm^{-1}; FAB-MS m/z 463 [M+H]$^+$, HRMS (FAB$^+$) calcd for $C_{27}H_{50}O_2Si_2$ [M]$^+$ 462.3349, found 462.3342.

4.5.5. 5-(*Z*)-Non-2-enyl-benzene-1,3-diol (climacostol (1)). To a solution of **9** (87.7 mg, 0.19 mmol) in THF (1.1 mL) was added 70% HF·pyridine (0.19 mL) at 0 °C under an argon atmosphere. After the mixture was stirred for 1 h at 0 °C, the reaction temperature was gradually raised to 25 °C. The reaction mixture was added to saturated aqueous $NaHCO_3$, and extracted with diethyl ether. The organic layer was washed with 1 M HCl, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt, 1:1) to give climacostol (**1**) (38.3 mg, 86%). ^{1}H NMR and ^{13}C NMR spectra were identical with those of natural **1**; IR (nujol) 2980, 2960, 2880, 1615, 1480, 1390, 1350, 1320, 1170, 1020, 980, 850 cm^{-1}; LC/ESI-MS/MS (in negative mode) m/z 217 [M−2H−CH$_3$]$^-$, 203 [M−2H−C$_2$H$_5$]$^-$, 189 [M−2H−C$_3$H$_7$]$^-$, 175 [M−2H−C$_4$H$_9$]$^-$, 161 [M−2H−C$_5$H$_{11}$]$^-$, 147 [M−2H−C$_6$H$_{13}$]$^-$, 122 [M−H−CH=CHC$_6$H$_{13}$]$^-$, 108 [M−H−CH$_2$CH=CHC$_6$H$_{13}$]$^-$.

4.5.6. 1,3-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-non-2-ynyl-benzene (10). To a solution of **7** (211 mg, 0.28 mmol) in THF (5 mL) was added I_2 (0.50 g, 3.94 mmol) in THF (10 mL). After the mixture was stirred at 25 °C for 2 h, was added saturated aqueous $NaHCO_3$ and saturated aqueous $NaHSO_3$ at 0 °C, and extracted with diethyl ether. The organic layer was washed with brine, and dried over anhyd. $MgSO_4$. The solvent was removed under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/diethyl ether, 40:1) to give **10** (117 mg, 90%): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.46 (2H, d, J=2.2 Hz, H-4, 6), 6.19 (1H, t, J=2.2 Hz, H-2), 3.45 (2H, t, J=2.4 Hz, H-1′), 2.18–2.23 (2H, m, H-4′), 1.48–1.54 (2H, m, H-5′), 1.34–1.44 (2H, m, H-6′), 1.27–1.34 (4H, m, H-7′, 8′), 0.97 (18H, s, Si–C(CH$_3$)$_3$), 0.89 (3H, t, J=7.0 Hz, H-9′), 0.19 (12H, s, Si–CH$_3$).

4.5.7. 5-Non-2-ynyl-benzene-1,3-diol (11). To a solution of **10** (210 mg, 0.46 mmol) in THF (15 mL) dissolved in the Teflon vial was added 70% HF·pyridine (1.24 mL) dropwise using the Teflon syringe at 0 °C under an argon atmosphere. After the reaction temperature was gradually raised to 25 °C over 2 h, the reaction was quenched with saturated aqueous $NaHCO_3$, and was added 1 M HCl, and then extracted with AcOEt. The organic layer was dried over anhyd. $MgSO_4$, evaporated under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/AcOEt, 5:2) to give **11** (106 mg, quant): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.42 (2H, d, J=2.3 Hz, H-4, 6), 6.22 (1H, t, J=2.3 Hz, H-2), 4.79 (2H, s, OH), 3.47 (2H, t, J=2.4 Hz, H-1′), 2.21 (2H, tt, J=2.4, 7.1 Hz, H-4′), 1.48–1.56 (2H, m, H-5′), 1.37–1.43 (2H, m, H-6′), 1.24–1.36 (4H, m, H-7′, 8′), 0.89 (3H, t, J=7.0 Hz, H-9′).

4.5.8. 1,3-Bis-(*tert*-butyl-dimethyl-silanyloxy)-5-nonyl-benzene (12). To a solution of **9** (334 mg, 0.72 mmol) in MeOH (14 mL) was added 10% palladium-on-charcoal catalyst and the mixture was stirred under a hydrogen atmosphere at 25 °C for 1 h. The reaction mixture was filtered through the Celite pad, and the filtrate was evaporated under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/diethyl ether, 40:1) to give **12** (270 mg, 80%): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.28 (2H, d, J=2.1 Hz, H-4, 6), 6.16 (1H, t, J=2.1 Hz, H-2), 2.46 (2H, t, J=7.8 Hz, H-1′), 1.24–1.30 (14H, m, H-2′, 3′, 4′, 5′, 6′, 7′, 8′), 0.97 (18H, s, Si–C(CH$_3$)$_3$), 0.88 (3H, t, J=6.8 Hz, H-9′), 0.18 (12H, s, Si–CH$_3$).

4.5.9. 5-Nonyl-benzene-1,3-diol (13). To a solution of **12** (250 mg, 0.54 mmol) in THF (18 mL) dissolved in the Teflon vial was added 70% HF·pyridine (1.46 mL) dropwise using the Teflon syringe at 0 °C under an argon atmosphere. After the reaction temperature was gradually raised to 25 °C over 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, added 1 M HCl and then extracted with AcOEt. The organic layer was dried over anhyd. $MgSO_4$, evaporated under reduced pressure and then purified by silica gel column chromatography (*n*-hexane/AcOEt, 5:2) to give **13** (105 mg, 83%): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.25 (2H, d, J=2.2 Hz, H-4, 6), 6.18 (1H, t, J=2.2 Hz, H-2), 4.82 (2H, s, OH), 2.48 (2H, t, J=7.8 Hz, H-1′), 1.56 (2H, m, H-2′), 1.23–1.29 (12H, m, H-3′, 4′, 5′, 6′, 7′, 8′), 0.88 (3H, t, J=7.0 Hz, H-9′).

Acknowledgements

Thanks are due to the Analytical Division at Osaka City University for analyses, mass spectra measurements and 600 MHz NMR measurements. We thank Dr. M. Tavrovskaya, Institute of Cytology, Russian Academy of Sciences, St. Petersburg for the strain of *Climacostomum virens* and the strain of *D. margaritifer*. This work was partially supported by Grand-in-Aid for Scientific Research of MEXT Japan, and Italian MURST, which are gratefully appreciated.

References and notes

1. Tao, N.; Orlando, M.; Hyon, J.-S.; Gross, M.; Song, P.-S. *J. Am. Chem. Soc.* **1993**, *115*, 2526–2528.
2. (a) Checcucci, G.; Shoemaker, R. S.; Bini, E.; Cerny, R.; Tao, N.; Hyon, J.-S.; Gioffre, D.; Ghetti, F.; Lenci, F.; Song, P.-S. *J. Am. Chem. Soc.* **1997**, *119*, 5762–5763. (b) Maeda, M.; Naoki, H.; Matsuoka, T.; Kato, Y.; Kotsuki, H.; Utsumi, K.; Tanaka, T. *Tetrahedron Lett.* **1997**, *38*, 7411–7414. (c) Spitzner, D.; Hofle, G.; Klein, I.; Pohlan, S.; Ammermann, D.; Jaenicke, L. *Tetrahedron Lett.* **1998**, *39*, 4003–4006.
3. (a) Tartar, V. *The Biology of Stentor*; Pergamon: New York, 1961. (b) Giese, A. C. *Blepharisma*; Stanford University Press: California, 1973.
4. (a) Song, P.-S. *Biochim. Biophys. Acta* **1981**, *639*, 1–29. (b) Scevoli, P.; Bisi, F.; Colombetti, F.; Ghetti, F.; Lenci, F.; Pasarelli, V. *J. Photochem. Photobiol. B: Biol.* **1987**, *1*,

75–84. (c) Matsuoka, T.; Watanabe, Y.; Sagara, Y.; Takayanagi, M.; Kato, Y. *Photochem. Photobiol.* **1995**, *62*, 190–193.
5. (a) Miyake, A.; Harumoyo, T.; Iio, H. *Eur. J. Protistol.* **2001**, *37*, 77–88. (b) Harumoto, T.; Miyake, A.; Ishikawa, N.; Sugibayashi, R.; Zenfuku, K.; Iio, H. *Eur. J. Protistol.* **1998**, *34*, 458–470. (c) Terazima, M. N.; Iio, H.; Harumoto, T. *Photochem. Photobiol.* **1999**, *69*, 47–54. (d) Miyake, A.; Harumoto, T.; Salvi, B.; Rivola, V. *Eur. J. Protistol.* **1990**, *25*, 310–315.
6. Miyake, A.; Buonanno, F.; Saltalamacchia, P.; Masaki, M. E.; Iio, H. *Eur. J. Protistol.* **2003**, *39*, 25–36.
7. Masaki, M. E.; Harumoto, T.; Terazima, M. N.; Miyake, A.; Usuki, Y.; Iio, H. *Tetrahedron Lett.* **1999**, *40*, 8227–8229.
8. (a) Suzuki, Y.; Esumi, Y.; Hyakutake, H.; Kono, Y.; Sakurai, A. *Phytochemistry* **1996**, *41*, 1485–1489. (b) Suzuki, Y.; Esumi, Y.; Uramoto, M.; Kono, Y.; Sakurai, A. *Biosci. Biotech. Biochem.* **1997**, *61*, 480–486.
9. Bandyopadhyay, C.; Gholap, A. S.; Mamdapur, V. R. *J. Agric. Food Chem.* **1985**, *33*, 377–379.
10. 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.
11. (a) Arisawa, M.; Ohmura, K.; Kobayashi, A.; Morita, N. *Chem. Pharm. Bull.* **1989**, *37*, 2431–2434. (b) Warshawsky, A. M.; Patel, M. V.; Chen, T.-M. *J. Org. Chem.* **1997**, *62*, 6439–6440.
12. (a) Kira, K.; Isobe, M. *Tetrahedron Lett.* **2000**, *41*, 5951–5955. (b) Kira, K.; Tanda, H.; Hamajima, A.; Baba, T.; Takai, S.; Isobe, M. *Tetrahedron* **2002**, *58*, 6485–6492.
13. (a) Hosokawa, S.; Isobe, M. *Synlett* **1996**, 351–352. (b) Hosokawa, S.; Isobe, M. *Tetrahedron Lett.* **1998**, *39*, 2609–2612. (c) Shibuya, S.; Isobe, M. *Tetrahedron* **1998**, *54*, 6677–6698.
14. Abe, Y.; Mori, K. *Biosci. Biotech. Biochem.* **2001**, *65*, 2110–2112.
15. Kozubek, A.; Tyman, J. H. P. *Chem. Rev.* **1999**, *99*, 1–25.
16. Thomson, R. H. *Naturally Occurring Quinones*; 2nd ed.; Academic: New York, 1971; pp 576–633.
17. Miyake, A. *Jpn J. Protozool.* **2002**, *33*, 97–117, in Japanese.
18. (a) Hofle, G.; Pohlan, S.; Uhlig, G.; Kabbe, K.; Schumacher, D. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1495–1497. (b) Lueken, W.; Uhlig, G.; Kruppel, T. *J. Protozool.* **1989**, *36*, 350–353.
19. (a) Dini, F.; Guella, G.; Giubbilini, P.; Mancini, I.; Pietra, F. *Naturwissenschaften* **1993**, *80*, 84–86. (b) Ronald, A.; Raymond, L. *J. Am. Chem. Soc.* **2001**, *123*, 9455–9456.
20. (a) Guella, G.; Dini, F.; Pietra, F. *Helv. Chim. Acta* **1995**, *78*, 1747–1754. (b) Rosini, G.; Laffi, F.; Marotta, E.; Pagani, I.; Righi, P. *J. Org. Chem.* **1998**, *63*, 2389–2391.
21. Guella, G.; Dini, F.; Pietra, F. *Helv. Chim. Acta* **1996**, *79*, 439–448.
22. (a) Tyman, J. H. P. *Synthetic and Natural Phenols*; Elsevier: Amsterdam, 1996; pp 465–557. (b) Kozubek, A.; Zarnowski, R.; Stasiuk, M.; Gubernator, J. *Cell. Biol. Mol. Lett.* **2001**, *6*, 351–355.
23. (a) Lytollis, W.; Scannell, R. T.; An, H.; Murty, V. S.; Reddy, K. S.; Barr, J. R.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12683–12690. (b) Singh, U. S.; Scannell, R. T.; An, H.; Carter, B. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12691–12699.
24. Lee, J. S.; Cho, Y. S.; Park, E. J.; Kim, J.; Oh, W. K.; Lee, H. S.; Ahn, J. S. *J. Nat. Prod.* **1998**, *61*, 867–871.
25. Valcic, S.; Wachter, G. A.; Eppler, C. M.; Timmermann, B. N. *J. Nat. Prod.* **2002**, *65*, 1270–1273.
26. Terazima, M. N.; Iio, H.; Harumoto, H. *Jpn J. Protozool.* **2001**, *34*, 60.
27. Muto, Y.; Tanabe, Y.; Kawai, K.; Iio, H. *Jpn J. Protozool.* **2003**, *36*, 25–26.
28. Miyake, A. In *Sexual Interaction in Eukaryotic Microbes*; O'Day, D. H., Horgen, P. A., Eds.; Academic: New York, 1981; pp 95–129.