Biosci. Biotechnol. Biochem., **69** (2), 391–396, 2005

Synthesis and Algicidal Activity of (+)-Cyanobacterin and Its Stereoisomer

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Received October 7, 2004; Accepted November 11, 2004

(+)-Cyanobacterin, a photosynthesis inhibitor of freshwater cyanobacterium *Schytonema hofmanni*, was synthesized in 6 steps from a readily accessible chiral synthon 5*R*-5-(*l*-menthyloxy)-2(5*H*)furanone.

Key words: cyanobacterin; photosynthesis inhibitor; algicidal activity; allelopathy; short synthesis

Cyanobacterin (**1a**) is an allelopathic substance isolated from the freshwater cyanobacterium *Schytonema hofmanni* UTEX2349 by Mason *et al.*[1] in 1982. Its structure, involving the relative stereochemistry between C-2 and C-3 positions, was determined on the basis of spectroscopic informations in 1983 by Pignatello *et al.*,[2] and the proposed structure was confirmed by total synthesis and X-ray diffraction analysis of the racemate.[3] Later, in 1987, the 2*R*,3*R* absolute configuration was assigned to the naturally occurring (+)-**1a** on the basis of X-ray diffraction analysis by Gleason and Porwoll.[4] Cyanobacterin has been shown to be highly toxic to other cyanobacteria and a variety of eukaryotic algae by interrupting photosynthetic electron transport at a site presumably close to the primary electron acceptor in photosystem II.[5,6] A basic structure-activity relationship study was performed by Gleason's group,[7] in which the halogen atom on the aromatic ring and the hydroxyl group at C-3 position were shown to be essential for the activity.

The first total synthesis of racemic **1a** was achieved by Jong *et al.*[3] in 1984, and recently Haga *et al.*[8,9] reported an asymmetric synthesis of both enantiomers of a dechlorinated analog of cyanobacterin and formal total synthesis of (+)-**1a** by employing Evans's asymmetric alkylation as the key step.

In connection with our interest in the development of algicide against red-tide phytoplankton, we synthesized (+)-**1a** and its epimer **1b**, and their algicidal activity was evaluated against harmful algal blooms (HAB) microalgae.

Results and Discussion

Feringa's group developed a strategy for the synthesis of optically active 2,3-disubstituted-γ-butyrolactones based on tandem conjugate addition-alkylation[10] of readily accessible chiral synthon 5*R*-5-(*l*-menthyloxy)-2(5*H*)furanone (**2**),[11] and they have demonstrated this strategy to be profitable for the synthesis of chiral butanediols[12,13] and lignans.[14,15] We envisaged (+)-**1a** also to be synthesized using Feringa's strategy as the key step (Fig. 1).

The synthesis was commenced with stereoselective 1,4-addition of isopropyl anion (*i*-PrMgBr, TMSCl, $CuBr \cdot Me_2S$, HMPA, THF)[16] to (5*R*)-(*l*-menthyloxy)-2(5*H*)furanone (**2**) $[[\alpha]_D^{25}$ −133.5° (*c* 1.02, EtOH), lit.[11] $[\alpha]_D$ −136.4° (EtOH), 98% ee] prepared by photooxidation of furfural followed by condensation with *l*-menthol and optical resolution according to the reported procedure,[11] in which (4*R*,5*R*)-**3** was obtained exclusively in 51% yield. Alkylation of **3** (LDA, THF)

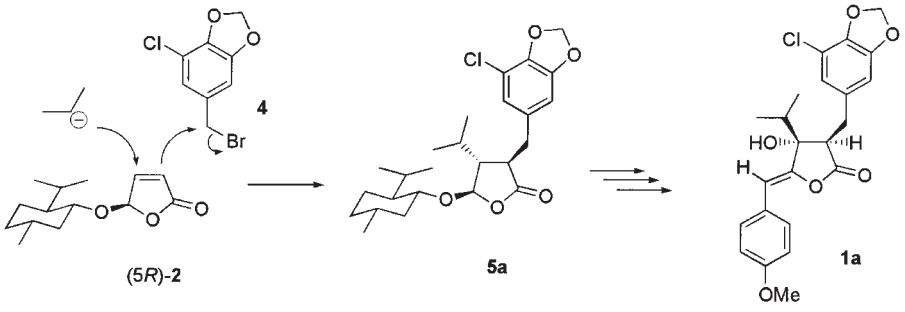

Fig. 1. Strategy for the Synthesis of (+)-Cyanobacterin.

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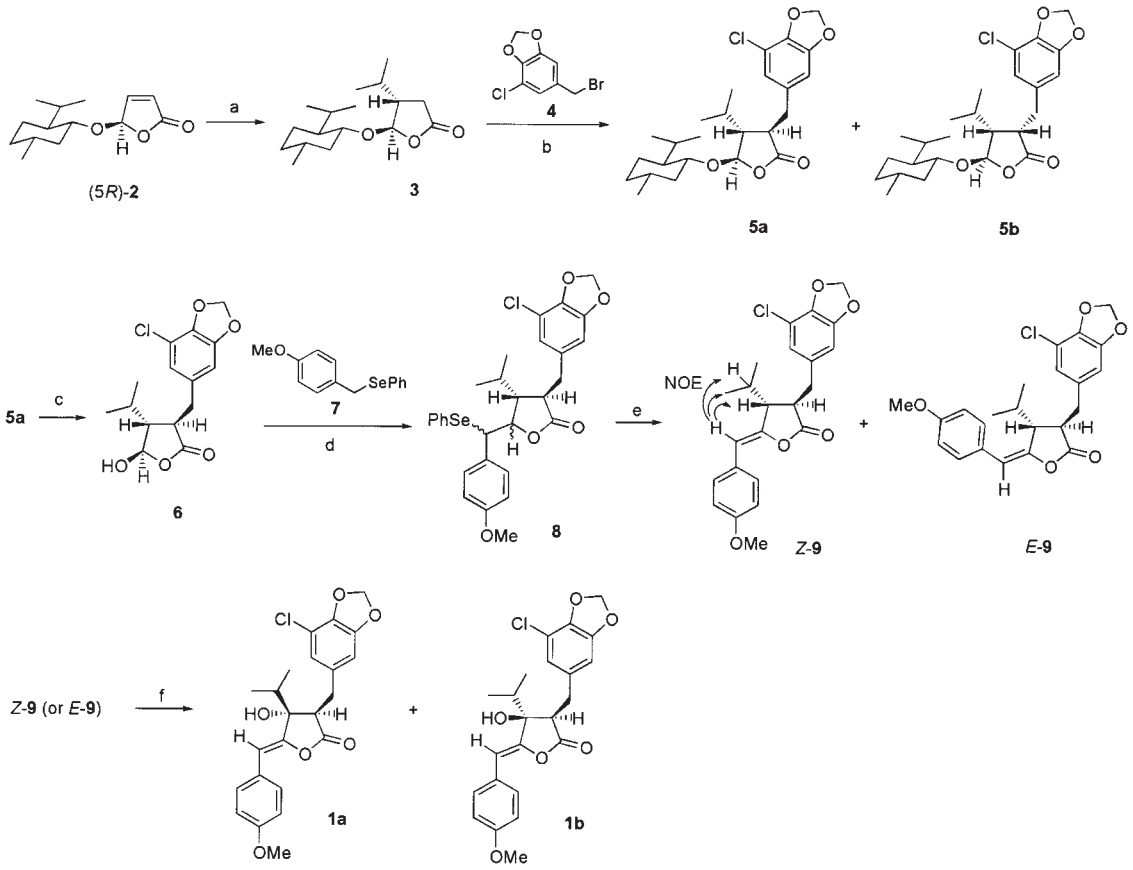

Scheme 1. Synthesis of (+)-Cyanobacterin (**1a**) and Its Epimer (**1b**).
Reagents: (a) *i*-PrMgBr, CuBr·SMe_2, TMSCl, HMPA, THF, −78 °C (34%); (b) LDA, THF, HMPA, −78 °C (69%); (c) KOH, aq. MeOH (86%); (d) LDA, THF, −78 °C, then *p*-TsOH, benzene, reflux; (e) aq. H_2O_2, CH_2Cl_2, rt (32%); (f) SeO_2, dioxane, 80 °C.

with 3-chloro-4,5-methylenedioxybenzyl bromide (**4**)[3] prepared in 5 steps from vaniline afforded 3,4-*trans* **5a** and 3,4-*cis* **5b** in a ratio of 89:11 in 69% combined yield. The chiral auxiliary was removed by alkaline hydrolysis (1 M KOH, MeOH), giving lactol **6** in 86% yield as a single stereoisomer, whereas an acid hydrolysis gave inferior results (CF_3COOH, CH_2Cl_2, rt, 24 h, 11%). The stereochemistry at the 5-position was assigned as *R* (4,5-*trans*) due to a coupling constant value ($J = 2.8$ Hz) between H-4 and H-5.

We then proceeded to incorporate the 4-methoxybenzylidene unit at the 5-position using selenide-selenoxide chemistry. The addition of the lithium carbanion generated from selenide **7** by treatment with LDA (THF) to lactol **6** followed by lactonization (cat. *p*-TsOH, benzene) afforded a diastereomeric mixture of the adduct **8**, which on treatment with hydrogen peroxide (CH_2Cl_2–H_2O) gave a separable mixture of 5*Z*- and 5*E*-benzylidenelactone *Z*-**9** and *E*-**9** in a ratio of 58:42 (based on NMR) in 32% combined yield. These stereoisomers were separated by chromatography on silica gel. The stereochemistry of these geometrical isomers was assigned on the basis of NOESY spectrum of *Z*-**9**, in which NOE correlation peaks between the olefinic proton (δ 5.42) and both methine protons at 4-C (δ 2.90–3.05) and methyl protons of the isopropyl moiety (δ 0.77–0.88) were observed.

Finally, the synthesis was accomplished by allylic hydroxylation of **9** with inversion of configuration at the 4-position. When *Z*-olefin **9** was treated with 3.6 equivalent of selenium dioxide in dioxane at 80 °C, (+)-**1a** and its epimer at 5-C (**1b**) were obtained in a ratio of **1a**:**1b** = 12:82 in 41% combined yield. A mechanism for the selenium dioxide-mediated allylic oxidation was first proposed by Sharpless *et al.*,[17,18] in which two consecutive pericyclic reactions, an initial ene reaction followed by a [2,3]-sigmatropic shift, are involved. Recently, the stereochemical aspects of the initial ene reaction and the whole reaction were clarified by a kinetic isotope effect study[19] and an *ab initio* study[20] respectively. A proposed mechanism for the reaction of *Z*- and *E*-**9** with selenium dioxide is shown in Fig. 2. The initial ene reaction of *Z*-**9** might proceed on the less hindered β-face of the molecule of **9** to form an allylselenic acid intermediate (**Z1**/**Z2**). In the subsequent [2,3]-sigmatropic shift, the transition state **Z2** leading to **1a** might be destabilized by the steric hindrance between the 4-methoxyphenyl moiety and the substituents at the 3-C and 4-C positions. Consequently, the reaction might proceed predominantly *via* the transition state **Z1**, and afford **1b** as the main product. On the contrary, *E*-**9** was expected to give **1a** as

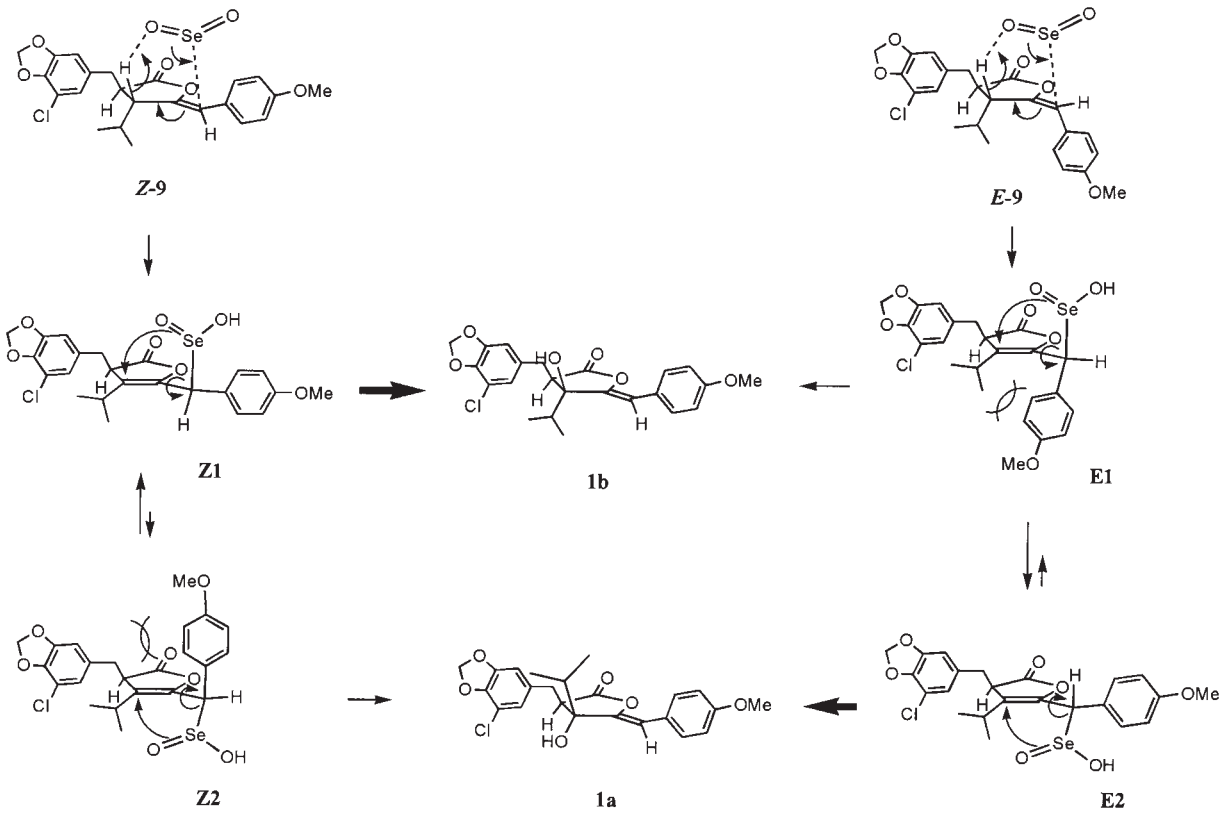

Fig. 2. Proposed Mechanism for the Selenium Oxide-Mediated Allylic Oxidation of *Z*- and *E*-**9**.

the main product, since the transition state **E2** leading to **1a** appeared to be more favorable than **E1**, which lead to **1b**. In fact, the reaction of *E*-**9** with selenium dioxide in dioxane at 80 °C for 24 h gave a mixture of **1a** and **1b** (**1a**:**1b** = 89:11. based on HPLC) in 19% combined yield with 71% recovery of *E*-**9**. Interestingly, no *E*-isomer of **1a** was formed in the reaction. The reaction of *E*-**9** required a longer reaction period than that of *Z*-**9**, probably due to a structural disadvantage for the first ene reaction, but the reaction at elevated temperature (refluxing temperature) caused dehydration of the alcohols to give an anhydro derivative of **1a** as the major product. The synthesized **1a** was identical in all respects with natural **1a**, including the optical rotation value [$[\alpha]_D$ +96° ($CHCl_3$), lit $[\alpha]_D$ +102° ($CHCl_3$)].

Synthesized (+)-**1a** and its epimer (−)-**1b** were briefly tested for algicidal activity against three harmful algal blooms (HAB) microalgae (Table 1). Both (+)-**1a** and (−)-**1b** showed algicidal activity as high as the typical photosystem II inhibitor DCMU against *Heterocapsa circularisquama* (Raphidophyceae) and *Chattonella marina* (Dinophyceae), while *Heterosigma akashiwo* (Raphidophyceae) was insensitive to them.

Table 1. Toxicity of Cyanobacterin (**1a**), Its Epimer **1b** and DCMU to Harmful Algal Bloom (HAB) Microalgae at a Dose of 10 µg/ml after 4 h, Mortality (%) to Control

	(+)-**1a**	(−)-**1b**	DCMU
Heterosigma akashiwo	9	7	14
Heterocapsa circularisquama	30	45	63
Chattonella marina	64	97	84

In conclusion, although it is necessary to improve the stereoselectivity and the chemical yield of individual steps, we have accomplished a short synthesis of (+)-cyanobacterin from the readily available chiral synthon.

Experimental

Melting points (mp) were uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 200, Gemini 300, or UNITY plus 500 instrument at nearly 200, 300, or 500 MHz respectively. EIMS and HREIMS were recorded on a JEOL JMS-DX303 spectrometer. ^{13}C NMR Spectra were recorded in $CDCl_3$ on a JEOL JNM AL-400 spectrometer at 100 MHz. IR spectra were recorded on a Perkin Elmer System 2000 spectrometer. Optical rotation was recorded on a Jasco DIP-370 polarimeter. Tetrahydrofuran (THF) and dichloromethane were distilled from sodium metal/benzophenone ketyl and phosphorous pentoxide respectively under an atmosphere of dry nitrogen prior to use.

*(4R,5R)-4-Isopropyl-5-menthyloxydihydro-2(3H)-furanone (**3**).* To a solution of isopropylmagnesium bromide prepared by slowly adding a solution of 2-bromopropane (0.52 ml, 5.54 mmol) in dry THF (10 ml)

to a stirred slurry of Mg powder (0.14 g, 5.94 mmol) in dry THF (5 ml) followed by refluxing for 40 min, were added successively a solution of $CuBr \cdot (CH_3)_2S$ (0.41 g, 0.20 mmol) in HMPA (1.65 ml, 9.50 mmol), $(CH_3)_3SiCl$ (1.00 ml, 7.90 mmol), and a THF (10 ml) solution of 5*R*-**2** (1.00 g, 3.96 mmol) at −78 °C. After 3 h at that temperature, the reaction was quenched by the addition of triethylamine (1.0 ml), diluted with hexane, and washed with water. The aqueous layer was extracted with hexane. The combined organic layer was washed with water, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed on silica gel eluted with hexane:EtOAc = 9:1 to give **3** (0.60 g, 2.02 mmol, 51%) as a colorless oil. $[\alpha]_D^{25}$ −2.0° (*c* 6.86, $CHCl_3$). NMR δ_H (300 MHz) 0.76–0.96 (15H, m, C*H*$_3$ × 5), 0.77–1.30 (4H, m, C*H*$_2$ × 2), 1.30–1.48 (1H, m, CH_3C*H*–), 1.59–1.83 (3H, m, C*H*$_2$ and $(CH_3)_2$C*H*–), 2.02–2.19 (3H, m, $(CH_3)_2$C*H*– and *i*-Pr–C*H*– × 2), 2.27 (1H, dd, *J* = 5.4, 17.8 Hz, 3-H), 2.73 (1H, dd, *J* = 8.8, 18.0 Hz, 3-H), 3.52 (1H, dt, *J* = 4.2, 10.5 Hz, C*H*–O), 5.44 (1H, d, *J* = 2.76 Hz, 5-H). NMR δ_C 15.6 (CH_3), 19.6 (CH_3), 19.9 (CH_3), 20.9 (CH_3), 22.3 (CH_3), 23.1 (CH_2–*C*H_2–CH), 25.4 (*C*H–*i*-Pr), 29.6 (*C*HMe_2), 31.5 (*C*HMe_2), 31.6 (3-C), 34.3 (CH_2–*C*H_2–CH), 39.8 (4-C), 42.9 (CH–*C*H_2–CH–O), 47.7 (*C*H–CH_3), 77.0 (*C*H–O), 103.9 (5-C), 176.2 (2-C). IR ν_{max} (KBr) cm^{-1}: 2960 (m), 1739 (s), 1268 (m), 1166 (s), 1116 (m), 1074 (m). Anal. Calcd. for $C_{17}H_{30}O_3$: C, 72.30; H, 10.71. Found: C, 72.00; H, 11.13%.

*(3R,4R,5R)-4-Isopropyl-5-menthyloxy-3[3-chloro-(4,5-methylenedioxyphenyl)-methyl]dihydro-2(3H)-furanone (**5a**).* To a cooled (−78 °C) solution of **3** (4.3347 g, 15.35 mmol) in THF (70 ml) was added dropwise 2*M* heptane/THF/ethylbenzene solution of LDA (Aldrich) (10.00 ml, 20.00 mmol) and the mixture was stirred for 1 h at −78 °C. A solution of 3-chloro-4,5-methylendioxybenzyl bromide (**4**) (4.2413 g, 17.00 mmol) in THF (40 ml) was added dropwise. After 1 h, HMPA (5.30 ml, 30.00 mmol) was added and the whole was stirred for another 1 h. The reaction was then quenched by 10% NH_4Cl solution (100 ml) and extracted twice with *t*-BuOMe. The extracts were combined, dried over Na_2SO_4, and concentrated. The oily residue was chromatographed on silica gel eluted with hexane: EtOAc = 9:1 to give a mixture of **5a** and **5b** (4.74 g, 10.53 mmol, 69%), which was separated by medium-pressure liquid chromatography on silica gel (Merck, LiChroprep Si60, benzene:EtOAc = 50:1).

5a, $[\alpha]_D^{25}$ −90.7° (*c* 0.28, $CHCl_3$). NMR δ_H (500 MHz) 0.78 (3H, d, *J* = 7.0 Hz, CH_3), 0.81 (3H, d, *J* = 5.5 Hz, CH_3), 0.82 (3H, d, *J* = 5.5 Hz, CH_3), 0.89 (3H, d, *J* = 7.0 Hz, CH_3), 0.94 (3H, d, *J* = 6.5 Hz, CH_3), 0.93–1.00 (4H, m, C*H*$_2$ × 2), 1.23 (1H, m, $(CH_3)_2$C*H*), 1.38 (1H, m, *i*-Pr–C*H*), 1.61–1.69 (3H, m, 4-$(CH_3)_2$C*H*, and C*H*$_2$), 1.89 (1H, m, 4-H), 2.01 (1H, m, Me–C*H*), 2.10 (1H, m, *i*-Pr–C*H*), 2.54 (1H, qui, *J* = 5.00, 4-H), 2.86 (1H, dd, *J* = 9.0, 14.0 Hz, 3-C*H*–Ar), 3.03 (1H, dd, *J* = 5.5, 14.0 Hz, 3-C*H*–Ar), 3.51 (1H, dt, *J* = 4.0, 10.5 Hz, C*H*–O), 5.41 (1H, s, 5-H), 6.02 (2H, s, O–C*H*$_2$–O), 6.60 (1H, d, *J* = 1.53 Hz, 2′-H), 6.67 (1H, d, *J* = 1.53 Hz, 6′-H). NMR δ_C 15.5 (CH_3), 20.4 (CH_3), 20.9 (CH_3), 21.2 (CH_3), 22.3 (CH_3), 23.1 (CH_2–*C*H_2–CH–*i*-Pr), 25.5 (*C*H–*i*-Pr), 27.3 (*C*HMe_2), 31.3 (*C*HMe_2), 34.1 (*C*H_2–Ar), 34.4 (CH–*C*H_2–CH_2), 39.2 (CH–*C*H_2–CH–O), 43.4 (*C*H–CH_3), 47.8 (4-C), 48.6 (3-C), 75.7 (CH–O), 99.0 (5-C), 101.0 (OCH_2O), 108.7 (2′-C), 113.4 (5′-C), 123.4 (6′-C), 123.3 (1′-C), 142.8 (4′-C), 148.4 (3′-C), 178.6 (2-C). IR ν_{max} (KBr) cm^{-1}: 2957 (m), 1778 (s), 1486 (s), 1428 (m), 1259 (s), 1048 (m), 940 (s). EI-MS *m*/*z* (%): 450 (M^+, 49), 311 (59), 226 (40), 224 (43), 169 (72), 139 (58), 138 (100), 83 (87). Found: C, 66.91; H, 7.52. Calcd. for $C_{25}H_{35}ClO_5$: C, 65.66; H, 8.04.

5b, NMR δ_H (300 MHz) 0.79–0.98 (15H, m, CH_3 × 5), 0.93–1.29 (4H, m, CH_2 × 2), 1.34–1.45 (1H, m, $(CH_3)_2$C*H*), 1.65–1.72 (2H, m, CH_2), 1.87–1.91 (1H, m, 4-$(CH_3)_2$C*H*), 1.99–2.05 (1H, m, 4-H), 2.10–2.14 (1H, m, Me–C*H*), 2.56 (1H, m, *i*-Pr–C*H*), 2.85–2.91 (1H, m, 3-C*H*–Ar), 3.07 (1H, dd, *J* = 5.5, 13.5 Hz, 3-C*H*–Ar), 3.51 (1H, dd, *J* = 4.52, 10.53 Hz, C*H*–O), 5.41 (1H, s, 5-H), 6.03 (2H, s, O–C*H*$_2$–O), 6.58 (1H, d, *J* = 1.53 Hz, 2′-H), 6.65 (1H, d, *J* = 1.53 Hz, 6′-H).

*(3R,4R,5R)-5-Hydroxy-4-isopropyl-3[3-chloro-(4,5-methylenedioxyphenyl)-methyl]dihydro-2(3H)-furanone (**6**).* To a solution of **5a** (1.00 g, 2.38 mmol) in MeOH (30 ml), 1 M KOH solution (5.0 ml, 5.0 mmol) was added, and the mixture was allowed to stand for 3 h at room temperature. The mixture was then acidified with conc. HCl and extracted twice with CH_2Cl_2. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated. The oily residue was chromatographed on silica gel eluted with hexane: EtOAc = 5:1 to give **6** (0.64 g, 2.05 mmol, 86% yield) as a colorless viscous oil. $[\alpha]_D^{25}$ −27.2° (*c* 2.14, $CHCl_3$). NMR δ_H (300 MHz) 0.82 (3H, d, *J* = 6.0 Hz, CH_3), 0.84 (3H, d, *J* = 6.8 Hz, CH_3), 1.68 (1H, m, 4-$(CH_3)_2$C*H*), 1.94 (1H, m, 4-H), 2.59 (1H, m, 3-H), 2.90 (1H, dd, *J* = 8.8, 14.0 Hz, 2-C*H*–Ar), 3.07 (1H, dd, *J* = 3.6, 14.0 Hz, 2-C*H*–Ar), 3.98 (1H, br-s, OH), 5.58 [1H, br-s (d, *J* = 2.8 Hz in $CDCl_3$–D_2O), 5-H], 6.01 (2H, s, O*CH*$_2$O), 6.63 (1H, d, *J* = 1.6 Hz, 2′-H), 6.70 (1H, d, *J* = 1.6 Hz, 6′-H). NMR δ_C 19.2 (CH_3), 19.5 (CH_3), 29.0 (*C*HMe_2), 36.8 (*C*H_2–Ar), 45.2 (4-C), 51.9 (3-C), 101.0 (5-C), 101.7 (OCH_2O), 108.2 (2′-C), 113.5 (5′-C), 122.9 (6′-C), 132.8 (1′-C), 143.0 (4′-C), 148.5 (3′-C), 178.5 (2-C). IR ν_{max} (KBr) cm^{-1}: 3391 (br), 2964 (m), 1756 (s), 1618 (m), 1502 (m), 1486 (s), 1429 (s), 1261 (s), 1195 (m), 1048 (s), 939 (s). EI-MS *m*/*z* (%): 312 (M^+, 40), 226 (100), 169 (72), 84 (14). HRMS *m*/*z* (M^+): Calcd. For $C_{15}H_{17}ClO_5$: 312.0765, Found: 312.0740.

*1-Methoxy-4-(phenylselenomethy)lbenzene (**7**).* Clean cut sodium (0.40 g, 18 mmol) was added portionwise to a solution of diphenyl diselenide (2.50 g, 8.01 mmol) in

dry THF (15 ml) at room temperature under a nitrogen atmosphere. The mixture was refluxed for 6 h, at which time all of the sodium had been consumed and orange crystals of sodium phenylselenoxide appeared. The mixture was cooled to room temperature and a solution of 4-methoxybenzyl chloride (2.60 g, 16.0 mmol) in HMPA (3.07 ml, 17.6 mmol) was added. After refluxing for 4.5 h, the reaction mixture was cooled again to room temperature, poured into water, and extracted twice with CH_2Cl_2. After drying (Na_2SO_4) and removing the solvent, the oily residue was chromatographed on silica gel eluted with benzene:CH_2Cl_2 = 9:1 to give selenide **7** (3.55 g, 12.8 mmol, 80% yield) as white crystals. NMR δ_H (300 MHz) 3.78 (3H, s, ArOCH_3), 4.08 (2H, s, PhSeCH_2Ar), 6.78 (2H, d, J = 8.5 Hz, 3′-H), 7.13 (2H, d, J = 8.5 Hz, 2′-H), 7.22–7.30 (3H, m, Ph), 7.42–7.49 (2H, m, Ph).

*4-Isopropyl-5-[(4-methoxyphenyl)methylene]-3-[3-chloro(4,5-methylenedioxy-phenyl)methyl]dihydro-2(3H)-furanones (**9**).* To a cooled (−78 °C) solution of selenide **7** (1.30 g, 4.50 mmol) in dry THF (20 ml) was added a 2*M* heptane/THF/ethylbenzene solution of LDA (2.5 ml, 5.0 mmol), and the mixture was stirred for 1 h. A solution of **6** (0.64 g, 2.10 mmol) in THF (10 ml) was added dropwise and the whole was stirred for 1 h before being quenched with a 1 M HCl solution (20 ml). The organic layer was separated and the aqueous layer was extracted with EtOAc. Combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was dissolved in benzene (50 ml) containing a catalytic amount of *p*-TsOH, and refluxed for 30 min. The mixture was concentrated and the residue was chromatographed on silica gel eluted with toluene to give a diastereomeric mixture of **8**, which was used for the next step without further purification.

To a cooled (0 °C) and stirred solution of the crude selenide **8** in CH_2Cl_2 (30 ml), was added 30% aqueous H_2O_2 (30 ml). The two phase system was gradually warmed to room temperature over 2 h and the reaction was quenched by the addition of a 10%Na_2SO_3 solution (30 ml) and a 5%$NaHCO_3$ solution (30 ml). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and concentrated. The oily residue was chromatographed on silica gel eluted with hexane: EtOAc = 5:1 to give an *E*/*Z* mixture of **9** [0.27 g, 6.65 mmol, 32% overall yield from **6**, *E*:*Z* = 42:58 (based on NMR)]. The geometrical isomers were separated using silica gel TLC (Merck silica gel 60 F_{254}) developed with benzene:EtOAc = 50:1.

Z-**9**: NMR δ_H (300 MHz) 0.78–0.88 (6H, m, $CH_3 \times 2$), 1.79–1.89 (1H, m, 4-$(CH_3)_2$CH), 2.70–2.79 (2H, m, 3-CH_2Ar), 2.90–3.05 (1H, m, 4-H), 3.12–3.25 (1H, m, 3-H), 3.81 (3H, s, ArOCH_3), 5.42 (1H, s, C=CHAr), 6.02 (2H, s, OCH_2O), 6.59 (1H, d, J = 1.44 Hz, 2′-H), 6.67 (1H, d, J = 1.44 Hz, 6′-H), 6.85 (2H, d, J = 2.01 Hz, 3″-H), 7.49 (2H, d, J = 2.01 Hz, 2″-H). Found: C, 66.58; H, 5.52. Calcd for $C_{23}H_{23}ClO_5$: C, 66.65; H, 5.59.

E-**9**: NMR δ_H (300 MHz) 0.71 (6H, dd, J = 6.90, 22.62 Hz, $CH_3 \times 2$), 1.90–2.04 (1H, m, 4-$(CH_3)_2$CH), 2.75–2.77 (2H, m, 3-CH_2Ar), 2.95–3.09 (1H, m, 4-H), 3.16–3.27 (1H, m, 3-H), 3.80 (3H, s, ArOCH_3), 6.01 (2H, s, OCH_2O), 6.25 (1H, s, C=CHAr), 6.65 (1H, d, J = 1.59 Hz, 2′-H), 6.69 (1H, d, J = 1.59 Hz, 6′-H), 6.87 (2H, d, J = 0.85 Hz, 3″-H), 7.00 (2H, d, J = 8.85 Hz, 2″-H). NMR δ_C 16.4 (CH_3), 19.3 (CH_3), 29.4 (CHMe$_2$), 37.3 (CH_2–Ar), 43.8 (4-C), 47.3 (3-C), 55.3 (OCH_3), 101.8 (OCH_2O), 107.8 (2′-C), 107.9 (C=CH–Ar), 113.8 (5′-C), 114.2 (3″-C and 5″-C), 123.0 (6′-C), 126.2 (1″-C9, 128.9 (2″-C and 6″-C), 132.0 (1′-C), 144.9 (4′-C), 148.8 (*C*=CH–Ar), 152.2 (3′-C), 158.3 (4″-C), 177.8 (2-C). IR ν_{max}(KBr) cm^{-1}: 2961 (m), 1790 (s), 1674 (m), 1610 (m), 1514 (s), 1486 (s), 1429 (m), 1259 (s), 1123 (s), 1046 (s), 939 (m).

*4-Hydroxy-4-isopropyl-5-[(4-methoxyphenyl)methylene]-3-[3-chloro-(4,5-methylenedioxyphenyl)methyl]dihydro-2(3H)-furanone (**1a**, **1b**).* A solution of *Z*-**9** (59 mg, 0.142 mmol) and SeO_2 (57 mg, 0.510 mmol) in dioxane (15 ml) was heated at 80 °C for 1 h. The solution was diluted with EtOAc, washed with water, dried (Na_2SO_4), and concentrated. The residue was purified using silica gel TLC (Merck silica gel 60F_{254}) developed with toluene:EtOAc = 50:1 to give **1a** (3.0 mg, 0.0023 mmol, 5%) and **1b** (21.6 mg, 0.0523 mmol, 36%).

1a: $[\alpha]_D^{27}$ +96.6° (*c* 0.058, $CHCl_3$). NMR δ_H (500 MHz) 0.91 (3H, d, J = 5.83 Hz, CH_3), 1.09 (3H, d, J = 5.83 Hz, CH_3), 1.85 (1H, s, OH), 2.18 (1H, qui, J = 6.64 Hz, 4-$(CH_3)_2$CH), 2.89 (1H, dd, J = 13.85, 6.06 Hz, 3-CHAr), 3.10–3.18 (2H, m, 3-CHAr and H-3), 3.82 (3H, s, ArOCH_3), 5.71 (1H, s, C=CHAr), 6.02 (2H, s, OCH_2O), 6.77 (1H, d, J = 1.60 Hz, 2′-H), 6.82 (1H, d, J = 1.60 Hz, 6′-H), 6.88 (2H, d, J = 8.80 Hz, 3″-H), 7.53 (2H, d, J = 8.80 Hz, 2″-H). IR ν_{max} (KBr) cm^{-1}: 3500 (br), 1807 (m), 1682 (m), 1505 (s), 1487 (s), 1251 (s), 1046 (s), 939 (m). HRMS *m*/*z* [(M − H_2O)$^+$]: Calcd. for $C_{23}H_{21}ClO_5$: 412.1078, Found: 412.1086.

1b: $[\alpha]_D^{27}$ −22.8° (*c* 1.0, $CHCl_3$); NMR δ_H (300 MHz) 0.97 (6H, d, J = 6.3 Hz, $CH_3 \times 2$), 1.95 (1H, s, OH), 2.00 (1H, m, 4-$(CH_3)_2$CH), 2.82–2.88 (2H, m, 3-CH_2Ar), 3.10 (1H, m, H-3), 3.82 (3H, s, ArOCH_3), 5.70 (1H, s, C=CHAr), 5.96 (1H, d, J = 1.2 Hz, OCHO), 5.99 (1H, d, J = 1.2 Hz, OCHO), 6.69 (1H, d, J = 1.5 Hz, 2′-H), 6.76 (1H, d, J = 1.5 Hz, 6′-H), 5.96 (1H, d, J = 1.6 Hz, 3″-H), 7.52 (2H, d, J = 8.7 Hz, 2″-H).

Compounds **1a** and **1b** were also obtained in 19% combined yield (**1a**:**1b** = 89:11, based on HPLC) by the reaction of *E*-**9** with SeO_2 (5 eq.) in dioxane at 70 °C for 5 h.

Toxicity assay on HAB microalgae. The bioassay was performed according to Kakisawa's procedure[21] with slight modifications. In brief, microalgae used for the bioassay were cultivated in EV media at 20 °C for 5 d in

the dark for 12 h, and under light (37 μmol photons/$m^{-2}s^{-1}$) for 12 h per day prior to the bioassay. Each culture was diluted with sea water to a cell density of $0.11–0.22 \times 10^5$ cells/ml, and pipetted into a 26-well plastic microplate (1 ml/well). 2 μl of the methanolic sample solution of appropriate concentration was added to each of the three wells and another three wells was used as a control (2 μl of MeOH/well). After incubation for 2 h at 20 °C under illumination (37 μmol photons/$m^{-2}s^{-1}$), cell viability was counted under microscope (×20) observation.

Acknowledgment

We thank Professor Tatsuya Oda, who kindly provided us the strains of HAB microalgae. This work was supported by a grant-aid from the Japan Society for the Promotion of Science (no. 13660202).

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