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Takashi KITAYAMA[a], Kaneyoshi YAMAMOTO[a], Ryutaro UTSUMI[a], Masahiro TAKATANI[a], Richard K. HILL[b], Yasushi KAWAI[c], Seiji SAWADA[d] & Tadashi OKAMOTO[a]

[a] Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University

[b] Department of Chemistry, University of Georgia

[c] Institute for Chemical Research, Kyoto University

[d] Kyoto University of Education

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Chemistry of Zerumbone. 2. Regulation of Ring Bond Cleavage and Unique Antibacterial Activities of Zerumbone Derivatives

Takashi KITAYAMA,[1,†] Kaneyoshi YAMAMOTO,[1] Ryutaro UTSUMI,[1] Masahiro TAKATANI,[1] Richard K. HILL,[2] Yasushi KAWAI,[3] Seiji SAWADA,[4] and Tadashi OKAMOTO[1]

[1]*Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan*
[2]*Department of Chemistry, University of Georgia, Athens, Georgia 30602, U.S.A.*
[3]*Institute for Chemical Research, Kyoto University, Uji 611-0011, Japan*
[4]*Kyoto University of Education, Fushimi, Kyoto 612-0863, Japan*

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Further investigation of the chemistry of the eleven-membered cyclic sesquiterpene, zerumbone, the major component of the wild ginger, *Zingiber zerumbet* Smith, has revealed a new selective epoxidation process, a further example of a novel Favorskii-initiated double ring contraction, and a regiospecific fragmentation of zerumbone dibromide derivatives. Several zerumbone derivatives were found to be selective inhibitors of the growth of Gram-positive bacteria.

Key words: zerumbone; ring cleavage; antibacterial activity; histidine protein kinase; two-component system

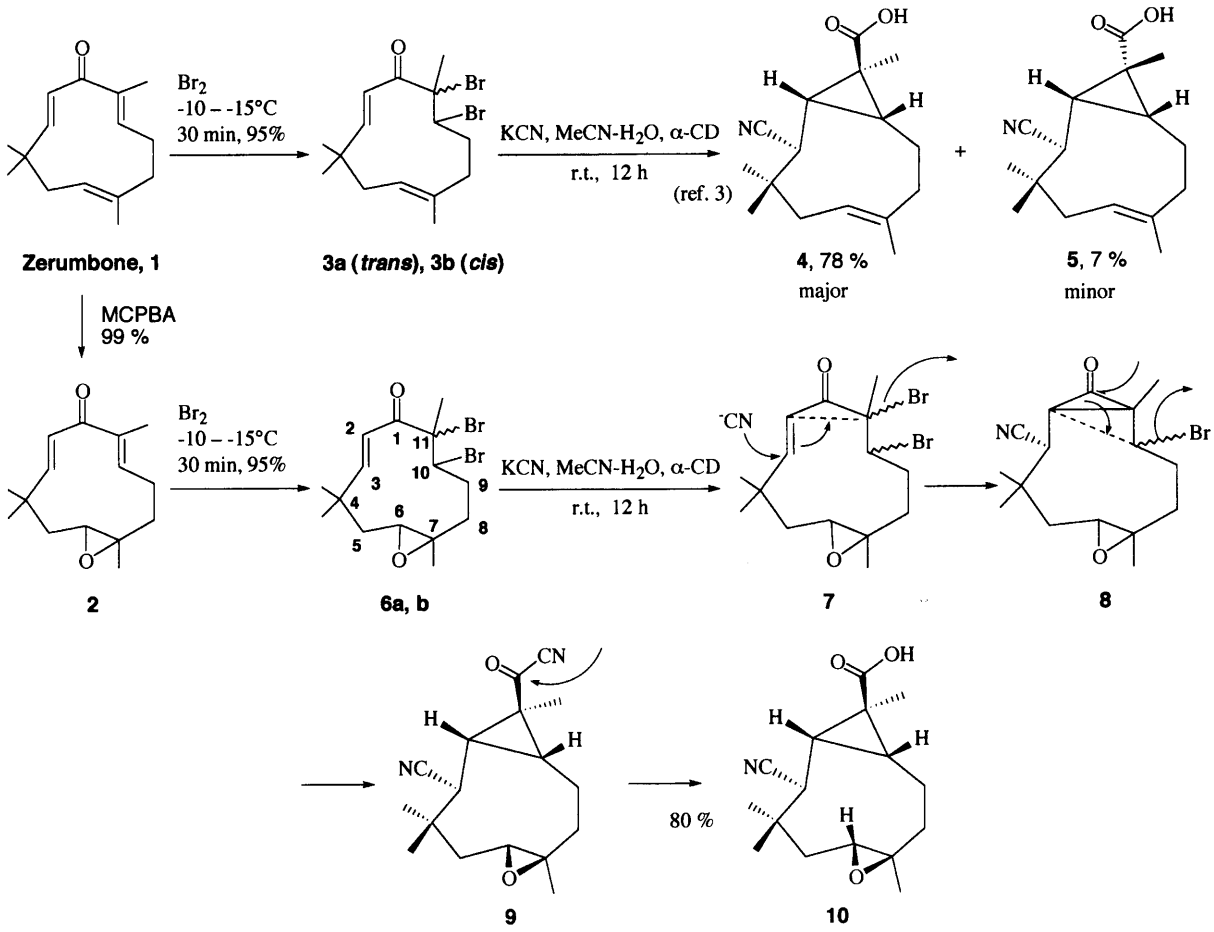
Zerumbone, (2*E*,6*E*,10*E*)-2,6,9,9-tetramethyl-2,6,10-cycloundecatrienone (**1**), is a monocyclic sesquiterpene found as the major component of the essential oil of the wild ginger, *Zingiber zerumbet* Smith.[1] Its chemistry was explored to a limited extent in the 1980s.[2] In our previous study,[3] we reported a simplified isolation procedure which makes zerumbone easily available in a large quantity and which allows a more thorough examination of the variety of addition, rearrangement, and transannular reactions possible for this interesting multifunctional structure. We have discovered a remarkable Favorskii rearrangement-intramolecular displacement sequence initiated by conjugate addition of cyanide to dibromozerumbone, leading to a bicyclo[7.1.0]decane ring system.[3] In this paper, we report our continued study of reagents which differentiate among the three double bonds and report a base-catalyzed fragmentation reaction which cleaved the zerumbone ring selectively at the C1-C2 bond. We also found that certain products derived from zerumbone selectively inhibited the growth of Gram-positive bacteria.

Results and Discussion

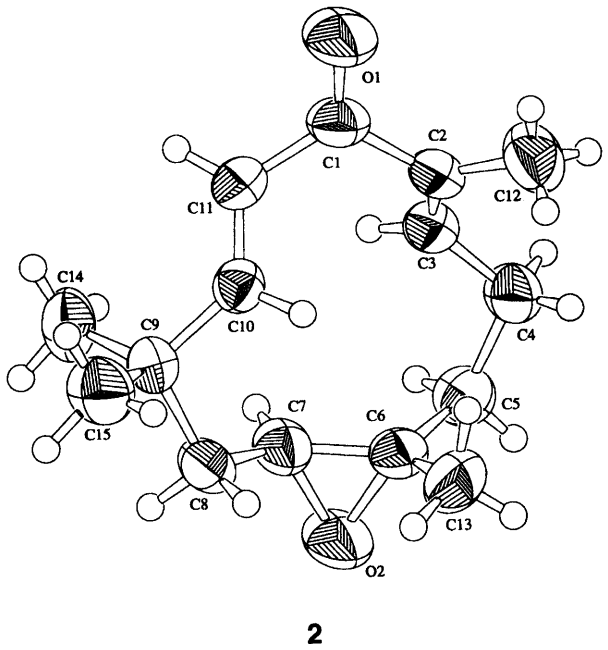
Epoxidation and intramolecular ring-contracting rearrangement

At −5°C, *m*-chloroperbenzoic acid reacted selectively with the isolated double bond of **1** (Scheme 1) to afford a quantitative yield of zerumbone oxide **2** (6,7-epoxy-2,6,9,9-tetramethyl-2,10-cycloundecadienone). The structure of **2** was determined by a single-crystal X-ray analysis (Fig. 1), which shows a conformation in which the two methyl groups at C2 and C6 are located on one face of the ring while the oxide oxygen lies on the opposite face, minimizing repulsion among these functional groups. Compared to the structure of zerumbone[4] itself, the C2-C3 double bond of **2** is significantly distorted from the plane of the dienone system. Judging solely from molecular models, the barrier to conformational flipping in **2** may be sufficient to induce an element of planar chirality, similar to that in the related ten-membered sesquiterpene, germacrone,[5] in addition to the conventional stereocenters. Analogous to the reaction with zerumbone (**1**→**3**→**4**+**5**),[3] one equivalent of bromine added regiospecifically at 0°C to the C2 conjugated double bond of oxide **2** gave a stereoisomeric mixture of 10,11-dibromo-6,7-epoxy-4,4,7,11-tetramethyl-2-cycloundecenone (**6a** and **6b**) in a 9:1 ratio and 95% yield. The structure of major isomer **6a**, the (10*R**, 11*R**) dibromide purified by recrystallization, was determined an by X-ray analysis (Fig. 2). Treatment of dibromide mixture **6a,b** with aqueous KCN at room temperature in the presence of α-cyclodextrin gave an 80% yield of ring-contracted carboxylic acid **10**, (1*R**, 2*S**, 5*R**, 6*R**, 9*R**, 10*S**)-2-cyano-5,6-epoxy-3,3,6,10-tetramethylbicyclo[7.1.0]-decane-10-carboxylic acid, paralleling the reaction of cyanide with zerumbone dibromide.[3] The structure of this Favorskii rearrangement product was con-

† To whom correspondence should be addressed. Tel: +81-742-43-7273(Ext 3302); Fax: +81-742-43-1445; E-mail: kitayama@nara.kindai.ac.jp

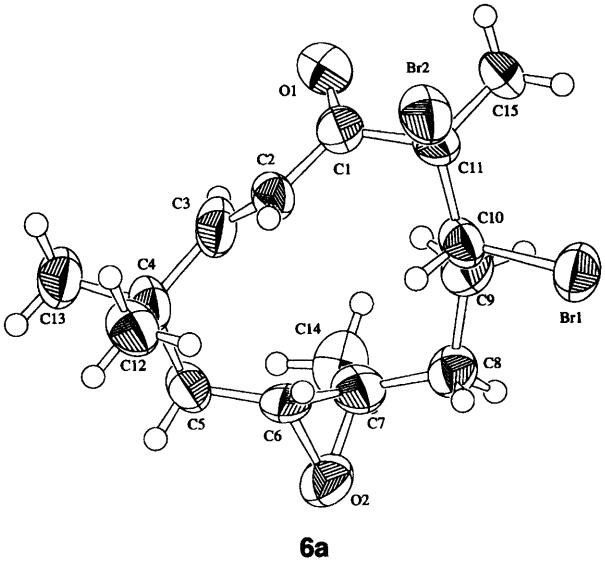


Scheme 1. Regulation of the Transfer of Ring Bonds.



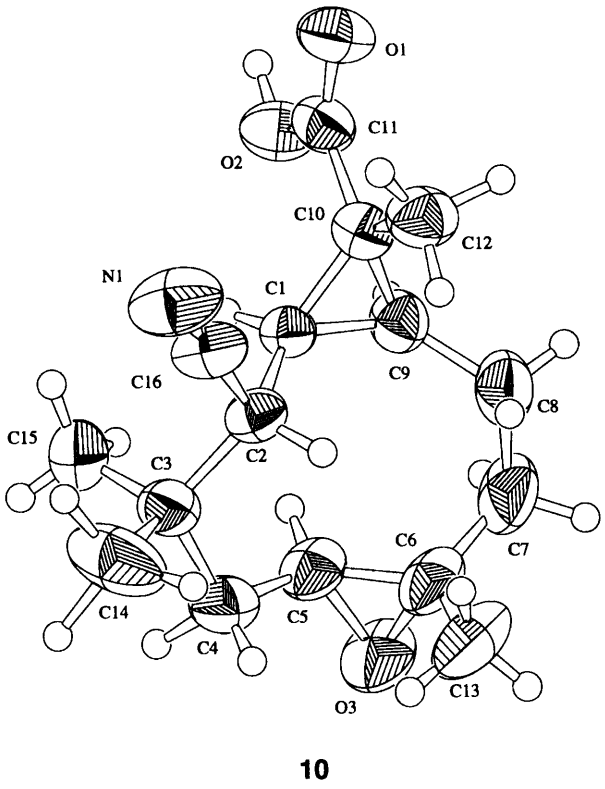
2

Fig. 1. ORTEP Drawing of the Crystal Structure of Epoxide **2**.



6a

Fig. 2. ORTEP Drawing of the Crystal Structure of Epoxydibromide **6a**.



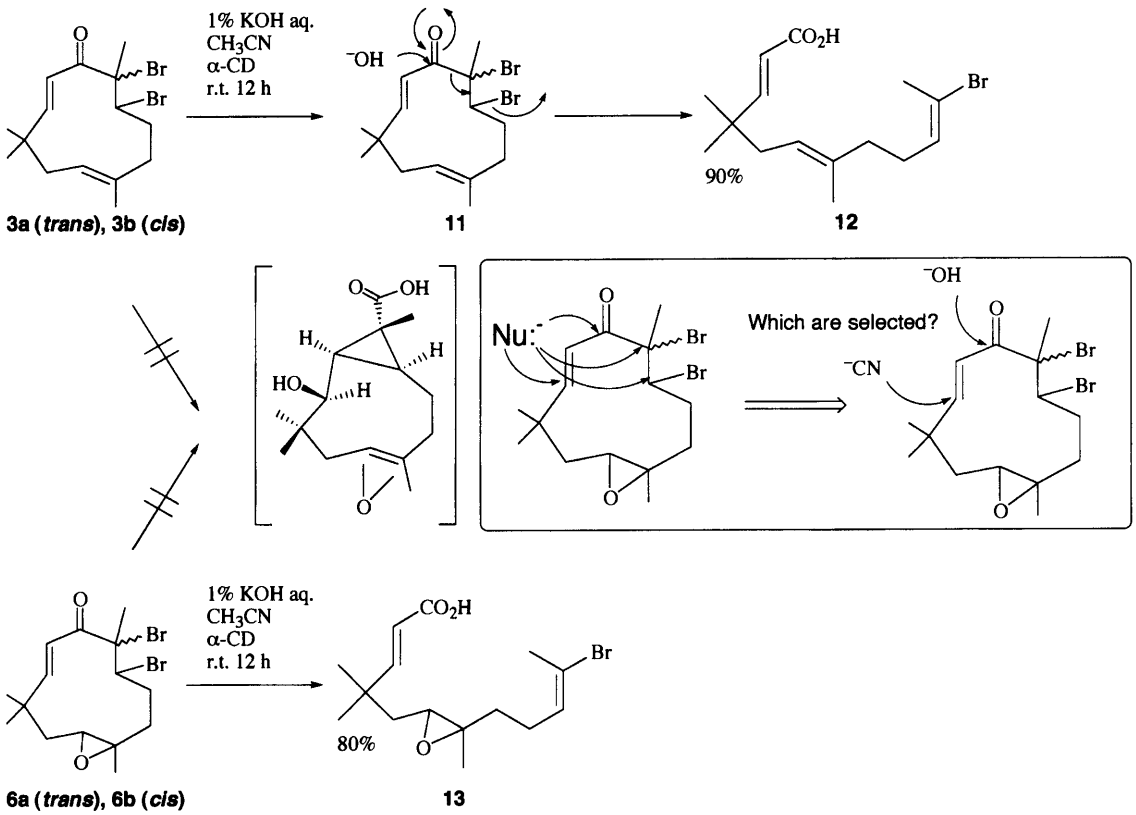
10

Fig. 3. ORTEP Drawing of the Crystal Structure of Bicyclic Carboxylic Acid **10**.

firmed by a single-crystal X-ray analysis (Fig 3).

Ring-opening reactions

Regioselective fragmentation of the C1-C2 bond in zerumbone was achieved by treating zerumbone dibromide **3** or zerumbone oxide dibromide **6** with aqueous KOH at room temperature in the presence of α-cyclodextrin (Scheme 2). Several courses of reaction with the hydroxide ion are open to these substrates, notably nucleophilic addition to the conjugated double bond initiating a Favorskii rearrangement, as have been observed for the reaction of **3** with cyanide.[3] Since hydroxide and cyanide ions have similar values for Reichart's nucleophilic parameters (4.75 *vs* 4.12),[6] they might have been expected to behave similarly toward **3**. The only reaction observed, however, was base-initiated fragmentation, leading to ring-opened carboxylic acids **12** and **13** in yields of 90% and 80%, respectively. The structures of the fragmentation products were clearly evident from their NMR spectra. The hydroxide-initiated fragmentation of β-haloketones has a number of precedents in monoterpene chemistry, including the cleavage of pulegone hydrochloride to citronellic acid,[7a] the fragmentation of 10-bromofenchone to γ-fencholenic acid,[7b] and the elegant application in a simple synthesis of *cis*-chrysanthemic acid,[7c] so it has not totally unexpected in the present case. Nevertheless, it is instructive to find that the



Scheme 2. Regulation of the Cleavage of Ring Bonds.

selection of a nucleophile could completely change the reaction course. This fragmentation reaction selectively cleaved the C1-C2 bond of zerumbone, and the Favorskii reaction already discussed selectively cleaved the C1-C11 bond (although maintaining a cyclic system). The isolated C6-C7 double bond was cleaved selectively by ozonolysis (unpublished observation from our lab.), allowing to date three different double bonds of the zerumbone skeleton to be selectively severed.

Antibacterial activity of the zerumbone derivatives

The rhizomes of *Zingiber zerumbet* Smith are employed as important materials of the traditional medicine, "JAMU", in Indonesia.[8a] Since zerumbone, the principal component, shows a variety of physiological effects, *e.g.* anti-cancer,[8b] HIV inhibitory,[9] anti-inflammatory,[10] plant growth regulatory,[11] and Epstein-Barr virus-inhibitory,[12] it was anticipated that the zerumbone derivatives might exhibit useful biological activity. On the other hand, bacteria contain multiple signal transduction systems, each comprising a histidine protein kinase (sensor kinase) and its cognate response regulator. These signal transduction systems are intimately involved in the maintenance of bacterial homeostasis and the expression of virulence determinants, including vancomycin resistance, in response to external and internal environmental stimuli.[13,14] Two-component systems containing histidine protein kinases essential for the growth of several bacteria have recently been reported: *yycF* and *yycG* of *Bacillus subtilis*, and *Staphylococcus aureus*. Such histidine protein kinases are considered ideal targets for drug-resistance,[15,16] because proteins similar in sequence have been identified in yeast, fungi and plant, but not in animal cells.

When the zerumbone derivatives synthesized in this study were tested as inhibitors of the autophosphorylation of two histidine protein kinases, Env Z and Pho Q, only ring-opened acid **12** selectively inhibited these kinases (Fig. 4). The effect of zerumbone derivatives as growth inhibitors of a Gram-positive bacterium (*Bacillus subtilis* 168) and a Gram-negative bacterium (*Escherichia coli* K-12, MC4100) was also investigated.[17] Compounds **4**, **10**, **12** and **13** all inhibited the growth of *B. subtilis* 168, the ring-opened acids **12** and **13** particularly showing potent activities, but none showed any effect on *E. coli* MC4100 (Fig. 5). Although we analyzed the autophosphorylation of histidine kinases EnvZ and PhoQ with 100 zerumbone derivatives that possessed the cyclic structure, we observed no inhibitor. In this study, a new halo-olefinic acid, **12**, that had been synthesized by C1 and C2 bond cleavage was newly identified to be an inhibitor of histidine kinases as well as

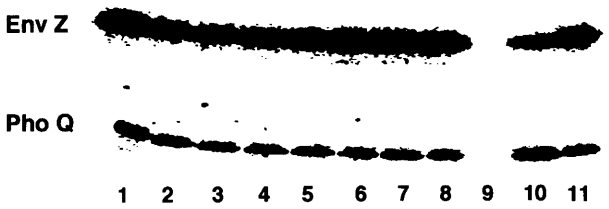


Fig. 4. Inhibition of the Autophosphorylation Activity of Two Histidine Kinases by Zerumbone Derivatives.

Kinases Pho Q and Env Z were autophosphorylated as described in the Experimental section after incubating with 500 mg/ml of a zerumbone derivative (lane 1, **1**; lane 2, **2**; lane 3, **3a**; lane 4, **3b**; lane 5, **4**; lane 6, **6a**; lane 7, **5**; lane 8, **10**; lane 9, **12**; lane 10, **13**; lane 11, DMSO). The autophosphorylated kinases were analyzed by SDS-PAGE. The gel was washed with a solution of methanol, acetic acid, and water (45:15:40, v/v, respectively), dried, and then analyzed by BAS1000 Mac (Fuji Film Co., Japan).

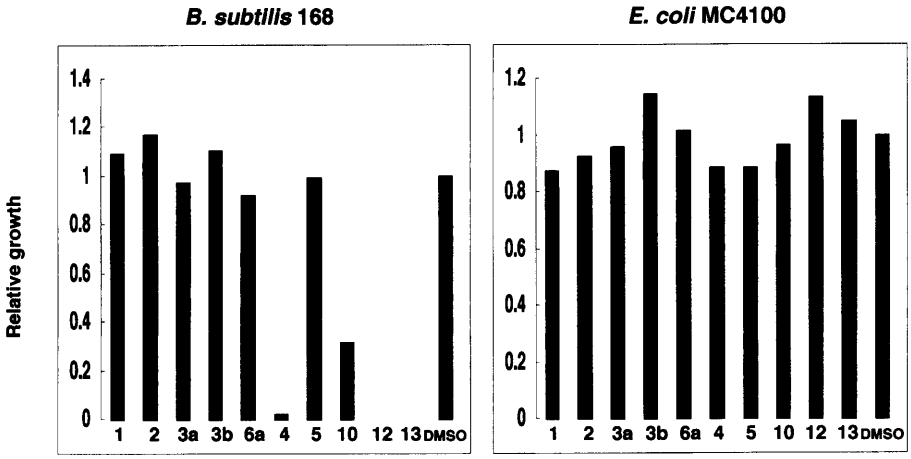


Fig. 5. Antibacterial Activities against *B. subtilis* 168 and *E. coli* MC 4100.

E. coli MC4100 and *B. subtilis* 168 were grown overnight at 37°C in an LB medium as described previously.[16] The cultures were diluted into a fresh LB medium in the presence or absence (control) of a zerumbone derivative (compounds **1**, **2**, **3a**, **3b**, **6a**, **4**, **5**, **10**, **12** and **13** each at 500 μg/ml) and 10^4 cells/ml and grown for 20 h. The optical density of each culture was then measured at 595 nm with a spectrophotometer (Shimadzu UV-1200). The ordinate indicates the relative growth compared with OD595 in the absence of the drugs.[15]

an antibacterial agent against *B. subtilis* 168.

B. subtilis 168 is well known to possess the two-component system, YycG (histidin kinase)-YycF (regulator), that is essential for its growth, but *E. coli* MC4100 is not. Yamamoto *et al.* have found that **12** also inhibited the autophosporylation of YycG (IC_{50}, 750 μM).[17] These facts indicate that compound12 blocked the growth of *B. subtilis* 168 by inhibiting YycG.

Experimental

General methods. NMR spectra were obtained at 270 MHz for ^1H and 68 MHz fof ^{13}C in $CDCl_3$ with TMS as the internal standard unless otherwise noted. Mass spectra were recorded at 70 eV, and high-resolution mass spectra (HRMS) were obtained by direct injection. Analytical and preparative HPLC was run in a column of Wakosil-II 5C18 HC (150×8 mm) or Wakosil 5C18 HC (300×8 mm), monitoring at 215 nm and eluting with a mixture of acetonitrile and water (7: 3), unless otherwise noted.

(6R,7R*)-Epoxy-2,6,9,9-tetramethyl-2,10-cycloundecadien-1-one (Zerumboneoxide)* (**2**). A solution of *m*-chloroperbenzoic acid (MCPBA, 2.0 g, 12 mmol) in methylene chloride (50 ml) was gradually added at −5°C to a stirred solution of **1** (2.2 g, 10 mmol) in the same solvent (30 ml). The mixture was stirred for 3 h, and a sodium carbonate solution (20 ml, 5%) was added. The organic layer was separated, dried over anhyd. Na_2SO_4, and concentrated under reduced pressure to yield a crystalline solid. The solid was recrystallized in hexane: ethyl acetate (EtOAc) to afford zerumbone epoxide 2, 2.3 g, 99%.

Mp 96.0–96.5°C; IR (KBr) cm^{-1}: 1657, 1263; ^1H-NMR δ: 1.10 (s, 3H, CH_3-11), 1.22 (s, 3H, CH_3-3), 1.31 (s, 3H, CH_3-11), 1.27-1.36 (m, 1H, H-4), 1.45 (dd, 1H, J=11.3 and 14.0 Hz, H-1), 1.85 (s, 3H, CH_3-7), 1.93 (d, 1H, J=14.0 Hz, H-1), 2.26–2.43 (m, 3H, H at C3 and 2H-4), 2.72 (dd, 1H, J=1.4 and 11.3 Hz, H-2), 6.07–6.12 (m, 3H, H−6, 9, and 10); ^{13}C-NMR δ: 12.3 (CH_3-7), 15.8 (CH_3-3), 23.9 (CH_3-11), 24.8 (5), 30.0 (CH_3-11), 36.1 (11), 38.2 (4), 42.8 (1), 61.2 (3), 62.8 (2), 128.6 (9), 139.3 (7), 147.6 (6), 159.3 (10), 202.3 (8). Elemental analysis. Found: C, 76.96; H, 9.41%. Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46%.

Crystallographic studies on zerumboneoxide (**2**). A colorless prismatic crystal, 0.60×0.60×0.40 mm, monoclinic, space group *C2/c* (no. 15), a=22.146(1), b=9.742(2), c=15.609(5) Å, β=125.002(3)°, V=2758.6(5) Å^3, Z=8, D_c=1.128 g/cm^3, μ (Cu Kα)=5.73 cm^{-1}, was used for data collection. The intensity data were measured on a Rigaku AFC7R diffractometer, using Cu-Kα radiation at a temperature of 20°C by the ω-2θ scan technique. The structure was solved by direct methods (SIR92)[18] and expanded by the Fourier technique (DIRDIF94).[19] All the calculations were performed by the teXsan crystallographic software package. The final cycle of full-matrix least-squares refinement was based on 2049 observed reflections ($I>1.50\sigma$(I)) and 243 variable parameters to give R=0.056 and Rw=0.088. The value for the goodness of fit indicator was 1.78.

(6R,7R*,10R*,11R*)-Dibromo-6,7-epoxy-4,4,7,11-tetramethyl-2-cycloundecen-1-one* (**6**). Bromine (0.75 g, 4.7 mmol) in CCl_4 (10 ml) was added dropwise at −15 to −10°C to a stirred solution of **2** (1.0 g, 4.3 mmol) in CCl_4 (5 ml). The final addition of bromine was titrated by following the change of the C11 proton in **2** by ^1H-NMR. The solution was concentrated under reduced pressure to afford crystalline dibromide **6** (1.69 g, 95%) in a diastereomer ratio of 9:1 by ^1H-NMR. The solid was recrystallized from EtOAc:hexane to afford the pure major isomer as a prismatic crystal, 1.44 g (85%).

IR (KBr) cm^{-1}: 2964, 1695, 1628; ^1H-NMR δ: 1.10 (d, 1H, J=9.0 Hz, H-8), 1.12 (s, 3H, CH_3-4), 1.20 (s, 3H, CH_3-7), 1.30 (dd, 1H, J=6.0 and 11.5 Hz, H-5), 1.30 (d, 1H, J=6.5 Hz, H-9), 1.32 (s, 3H, CH_3-4), 1.84 (s, 3H, CH_3-11), 1.92 (d, 1H, J=6.0 Hz, H-5), 2.10 (dd, 1H, 6.5 and 9.0 Hz, H-9), 2.20 (d, 1H, J=9.0 Hz, H-8), 2.62 (d, 1H, J=11.5 Hz, H-6), 4.21 (d, 1H, J=9.0 Hz, H-10), 6.43 (d, 1H, J = 16.0 Hz, H-3), 6.87 (d, 1H, J=16.0 Hz, H-2); ^{13}C-NMR δ: 17.1 (CH_3-4), 20.7 (CH_3-11), 23.5 (CH_3-4), 29.7 (CH_3-7), 31.3 (9), 36.7 (4), 36.8 (8), 39.0 (5), 59.9 (6), 60.7 (7), 62.4 (10), 69.1 (11), 124.3 (2), 154.7 (3), 192.8 (1). Elemental analysis. Found: C, 45.79; H, 5.64%. Calcd. for $C_{15}H_{22}O_2Br_2$: C, 45.71; H, 5.63%.

Crystallographic studies on (6R,7R*,10R*,11R*)-dibromo-6,7-epoxy-4,4,7,11-tetramethyl-2-cycloundecen-1-one* (**6a**). A colorless prismatic crystal, 0.21×0.17×0.61 mm, triclinic, space group *P1* (no. 2), a=8.7001(9), b=12.651(1), c=7.910(1) Å, α=98.560(10)°, β=106.294(8)°, γ=96.464(9)°, V=815.4(2) Å^3, Z=2, D_c=1.605 g/cm^3, μ (Cu Kα)=62.77 cm^{-1}, was used for data collection. The intensity data were measured with a Rigaku AFC7R diffractometer, using Cu-Kα radiation at a temperature of 22.0°C by the ω-2θ scan technique. The structure was solved by the direct method (SIR92)[18] and expanded by the Fourier technique (DIRDIF94).[19] All the calculations were performed by the teXsan crystallographic software package. The final cycle for full-matrix least-squares refinement was based on 1734 observed reflections ($I>1.50\sigma$(I)) and 173 variable parameters to give R=0.074 and Rw=0.122. The value for the goodness of fit indicator was 1.23.

(1R,2S*,5R*,6R*,9R*,10S*)-2-Cyano-5,6-epoxy-3,3,6,10-tetramethylbicyclo-[7.1.0]decane-10-*

carboxylic acid (**10**). A solution of the diastereomeric mixture of **6** (1.97 g, 5.0 mmol), KCN (0.75 g, 11.5 mmol), and α-CD (1.5 g) in acetonitrile and water (40 ml and 20 ml, respectively) was stirred for 12 h at 10 – 15°C. The alkaline solution was extracted with EtOAc (2 × 10 ml). The aqueous layer was acidified with HCl and extracted with EtOAc (3 × 10 ml). The organic layer was concentrated to a crystalline residue which was recrystallized from EtOAc:hexane to afford dominant diastereomer **10**, 1.31 g, 80%.

^{1}H-NMR δ: 1.16 (dd, 1H, J = 2.0 and 12.5 Hz, H-8), 1.22 (d, 1H, J = 6.0 Hz, H-7), 1.27 (s, 3H, CH_3-3), 1.30 (dd, 1H, 2.0 and 6.0 Hz, H-4), 1.32 (s, 3H, CH_3-6), 1.35 (s, 3H, CH_3-3), 1.36 (s, 3H, CH_3-10), 1.57 (dd, 1H, J = 6.0 and 12.5 Hz, H-9), 1.96 (dd, 1H, J = 6.0 and 12.0 Hz, H-4), 1.99 (dd, 1H, J = 6.0 and 10.0 Hz, H-1), 2.10 (d, 1H, J = 2.0 Hz, H-8), 2.15 (d, 1H, J = 10.0 Hz, H-2), 2.19 (d, 1H, J = 6.0 Hz, H-7), 3.06 (dd, 1H, J = 2.0 and 12.0 Hz, H-5); ^{13}C-NMR δ: 9.9 (CH_3-10), 17.6 (CH_3-3), 20.9 (8), 24.9 (CH_3 at 3), 26.1 (10), 30.0 (1), 32.5 (9), 33.3 (CH_3-6), 36.1 (3), 36.7 (2), 37.4 (7), 40.3 (4), 58.3 (5), 59.8 (6), 119.8 (CN), 180.8 (CO_2H). Elemental analysis. Found: C, 69.07; H, 8.40; N, 5.03%. Calcd. for $C_{16}H_{23}NO_3$: C, 69.29; H, 8.36; N, 5.05%.

Crystallographic studies on (1R,2S*,5R*,6R*,9R*,10S*)-2-cyano-5,6-epoxy-3,3,6,10-tetramethyl-bicyclo[7.1.0]decane-10-carboxylic acid* (**10**). A colorless needle crystal, 0.20 × 0.12 × 0.75 mm, monoclinic, space group $P2_1/n$ (no. 14), a = 8.972(1), b = 13.2663(8), c = 13.242(1) Å, β = 97.367(10)°, V = 1563.2(3) Å^3, Z = 4, D_c = 1.178 g/cm^3, μ(Cu Kα) = 6.51 cm^{-1}, was used for data collection. The intensity data were measured with a Rigaku AFC7R diffractometer, using Cu-Kα radiation at a temperature of 21.0°C by the ω-2θ scan technique. The structure was solved by the direct method (SIR92)[18] and expanded by the Fourier technique (DIRDIF94).[19] All the calculations were performed by the teXsan crystallographic software package. The final cycle of full-matrix least-squares refinement was based on 2044 observed reflections ($I > 1.50\sigma$(I)) and 274 variable parameters to give R = 0.054 and Rw = 0.088. The value for the goodness of fit indicator was 0.94.

(2E,6E,10EZ)-11-Bromo-4,4,7-trimethyl-2,6,10-dodecatrienoic acid (**12**). A solution of **3** (3.1 g, 8.2 mmol), KOH (0.83 g, 14.8 mmol), and α-CD (8.0 g, 8.2 mmol) in acetonitrile and water (40 ml and 40 ml, respectively) was stirred for 5h at room temperature. The mixture was diluted with water (50 ml) and acidified with HCl. The acidic solution was extracted with EtOAc (3 × 50 ml). The combined organic layers were successively washed with water (3 × 50 ml) and satd. aq. NaCl (2 × 50 ml), dried over anhyd. Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel with a 15:1 mixture of $CHCl_3$ and methanol as the eluent to afford **12** in a yield of 2.8 g, 90%.

IR (KBr) cm^{-1}: 3050, 1695, 1647; ^{1}H-NMR δ: 1.07 (s, 6H, 2CH_3-4), 1.58 (s, 3H, CH_3-7), 2.20 (s, 3H, CH_3-12), 2.05–2.16 (m, 6H, 2H – 5, 8, and 9), 5.10 (dd, 1H, J = 7.6 and 8.9 Hz, H-6), 5.73 (d, 1H, J = 15.9 Hz, H-3), 5.77 (m, 1H, H-10), 7.06 (d, 1H, J = 16.2 Hz, H-2); ^{13}C-NMR δ: 16.0 (CH_3-7), 23.2 (12), 25.9 (2CH_3-4), 28.1 (9), 37.9 (4), 39.0 (8), 40.0 (5), 117.1 (3), 119.2 (7), 120.8 (6), 131.7 (10), 136.6 (11), 161.0 (2), 172.7 (1); HRMS m/z: calcd. mass for $C_{15}H_{22}O_2Br$ (M-H), 313.0803; found, 313.0780.

(2E,6R,7R*,10EZ)-11-Bromo-6,7-epoxy-4,4,7-trimethyl-2,10-dodecadienoic acid* (**13**). A solution of **6** (1.2 g, 3.0 mmol), KOH (0.3 g, 5.4 mmol), and α-CD (2.8 g, 3.0 mmol) in acetonitrile and water (15 ml and 15 ml, respectively) was stirred for 5h at room temperature. The mixture was diluted with water (50 ml) and acidified with HCl. The acidic solution was extracted with EtOAc (3 × 50 ml). The combined organic layers were successively washed with water (3 × 50 ml) and satd. aq. NaCl (2 × 50 ml), dried over anhyd. Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to column chromatography over silica gel with a 15:1 mixture of $CHCl_3$ and methanol as the eluent to afford **13** in a yield of 0.95 g, 80%.

IR (KBr) cm^{-1}: 3070, 1697, 1651; ^{1}H-NMR δ: 1.15 (s, 3H, CH_3-4), 1.18 (s, 3H, CH_3-4), 1.23 (s, 3H (79%), CH_3 *trans*-12), 1.26 (s, 3H (21%), CH_3 *cis*-12), 1.59 (dt, 2H, J = 6.6 and 7.9 Hz, H-8), 1.68 (dd, 2H, J = 5.6 and 6.9 Hz, H-5), 2.10 (dt, 2H, J = 7.6 and 7.9 Hz, H *trans*-9), 2.23 (dt, 2H, J = 6.6 and 7.6 Hz, H *cis*-9), 2.27 (s, 3H, CH_3-7), 2.69 (dd, 3H, J = 5.6 and 5.9 Hz, H-6), 5.58 (dt, 1H, J = 1.3 and 6.6 Hz, H *cis*-10), 5.78 (dt, 1H, J = 1.3 and 7.6 Hz, H *trans*-10), 5.79 (d, 1H, J = 16.2 Hz, H-3), 7.07 (d, 1H, J = 15.8 Hz, H-2); ^{13}C-NMR δ: 16.6 (CH_3-12), 23.1 (CH_3-7), 25.1 (9), 26.1 (CH_3-4), 27.0 (CH_3-4), 36.5 (4), 37.7 (5), 40.6 (8), 59.4 (7), 59.9 (6), 118.1 (3), 120.0 (11), 131.0 (10), 158.9 (2), 171.7 (1); Elemental analysis. Found: C, 54.21; H, 7.13%. Calcd. for $C_{15}H_{23}O_3Br$: C, 54.39; H, 7.00%.

Autophosphorylation of EnvZ and PhoQ. PhoQ was purified by using the QIA express Ni-NTA protein purification system (Qiagen) as previously described.[16] Purified EnvZ was supplied by M. Inouye. One micromolar equivalent of PhoQ or EnvZ was incubated with 37 kBq of [γ-^{32}P]ATP in 50 mM tris-HCl at pH7.5, 50 mM KCl, and 10 mM $MgCl_2$ containing 2.5 mM cold ATP for 10 min at room temperature. To stop the reaction, a buffer of 120 mM Tris-HCl at pH6.8, 20% glycerol, 4%

SDS, 10% β-mercaptoethanol and 0.1% BPB was added.

Supporting information available: X-ray experimental details for **2**, **6a**, and **10**, tables of crystallographic data, atomic coordinates, anisotropic thermal parameters, bond lengths and angles, and torsion angles are available free of charge *via* the internet page for ordering information.

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