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Syntheses of Cerulenin and Its Analogs. II. Synthesis and Biological Activity of *dl*-Carbacerulenin, a Carbocyclic Analog of Cerulenin

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2,3-Epoxy-4-hydroxy-4-((*E*,*E*)-3,6-octadienyl)cyclopentanone (*dl*-carbacerulenin 5) was synthesized *via* the epoxyketones 15a and 15b as a mimic of the active form of the antibiotics cerulenin 1, a potent inhibitor of fatty acid synthetase (FAS). The monobenzyl ethers (12 and 13), synthetic intermediates of 15, were prepared by direct benzylation of the epoxycyclopentene (7). Inhibitory activity of synthesized 5 toward yeast FAS was less than that of cerulenin by a factor of 1000.

Keywords cerulenin; carbacerulenin; fatty acid synthetase inhibitor; 4-benzyloxy-2-cyclopentenol

The antibiotic cerulenin, (2*R*,3*S*)-2,3-epoxy-4-oxo-7,10-(*E*,*E*)-dodecadienamide (**1**),[1-3] is a potent inhibitor of fatty acid synthetase (FAS).[4] It specifically binds to the cysteine residue at the active site of β-ketoacyl thioester synthetase (condensing enzyme) and inhibits chain elongation in fatty acid biosynthesis.[4-6] The equilibrium of cerulenin between the two structures **1** and **2** lies very much in favor of **1** in aprotic solvents such as chloroform, and in favor of an epimeric mixture of **2a** and **2b** in a ratio of *ca.* 5 : 1 in protic solvents such as methanol.[7]

We previously reported that cerulenin did not react with cysteine methyl ester in chloroform solution, but, in buffer solutions, it reacted with both cysteine and cysteine methyl ester. The adducts **3a** and **3b** were formed, respectively, by attack of the SH-group upon C-2 of cerulenin.[7,8] The adduct **3a** was also isolated as a complete digestion product of the yeast FAS pretreated with ^{3}H-labeled cerulenin.[8]

Synthesized *N*,*N*-dimethylcerulenin (**4**), which is unable to cyclize, has only a low activity against FAS.[9] Our results described above together with the data on **4** led us to assume that the bioactive form of cerulenin might be the cyclic structure **2a**. In order to investigate this possibility, we planned to prepare carbacerulenin **5**, a simpler mimic of **2a**. This report describes the synthesis and yeast FAS-inhibitory activity of *dl*-carbacerulenin (**5**).

Synthesis The synthetic routes to *dl*-carbacerulenin **5** are shown in Chart 1. *trans*-2-Cyclopentene-1,4-diol (**8**) and *cis*-2-cyclopentene-1,4-diol (**9**) were obtained according to the procedure of Korach *et al.*[10,11] Peracetic acid oxidation of cyclopentadiene (**6**) followed by *in situ* hydrolysis gave a mixture of the 1,4-diols **8** and **9** in a ratio of 7 : 4 and the cyclopentene-3,4-diols **10** and **11**. By successive fractional distillation and silica gel column chromatography, the *trans*-diol **8** and a mixture of the diols **9** and **10** were obtained. The 1,4-diols were identified by comparison of their proton nuclear magnetic resonance (^{1}H-NMR) spectra with the reported data[12-14] and the structures of the 3,4-diols were elucidated from their mass spectral (MS)[15] and ^{1}H-NMR data. The diols **8** and **9** were then treated with one equivalent of NaH and benzyl chloride[16] to give the monobenzyl ethers, **12** and **13**, but the yield from **6** was only 10% based on peracetic acid used[17] because of the formation of dibenzyl ether. In order to improve the yield, direct conversion of epoxycyclopentene **7** to monobenzyl ethers was carried out. *In situ* treatment of the epoxide **7** with 5 eq of benzyl alcohol and 0.05 eq of *p*-toluenesulfonic

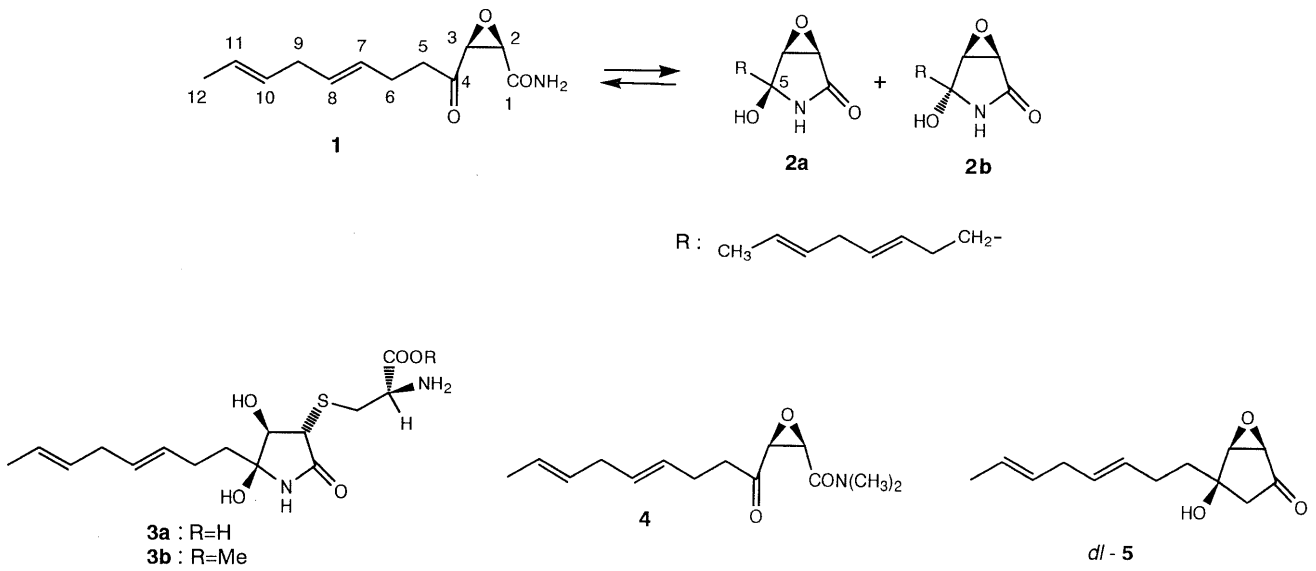

Fig. 1

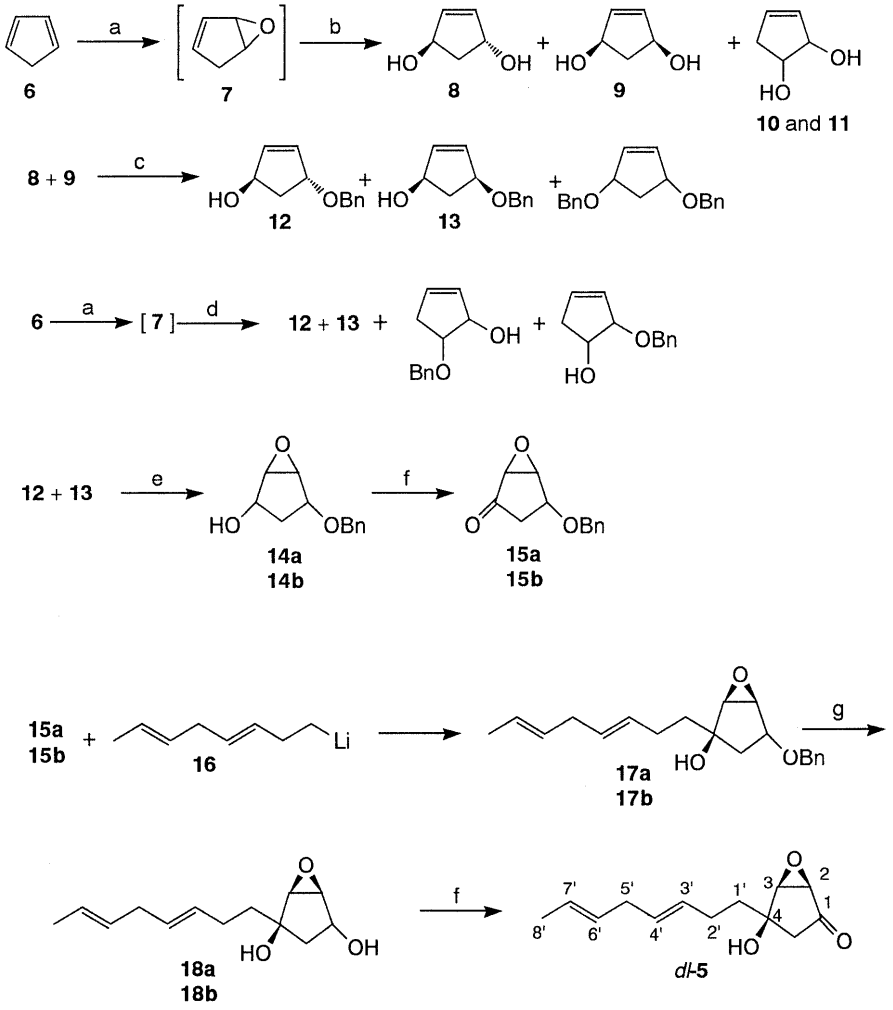

Chart 1

acid instead of hydrolysis gave a mixture of the desired monobenzylethers **12** and **13** (2 : 1) in 30% yield on the basis of peracetic acid used.[17] The mixture of the ethers **12** and **13** was oxidized with *m*-chloroperbenzoic acid (MCPBA) to give the epoxides **14a** and **14b** in 55% and 15% yields, respectively. Both epoxides showed ^{1}H-NMR signals at δ 3.53 (1H, m) and 3.57 (1H, m) due to the protons on the carbons bearing the epoxy group. Swern oxidation[18] of a mixture of **14a** and **14b** afforded the epoxy ketones **15a** and **15b** in 64% and 22% yields, respectively.

Epoxy ketones thus obtained were then treated with (*E,E*)-3,6-octadienyl lithium (**16**)[19,20] to give compounds **17a** and **17b** in 54% and 18% yields, respectively. Introduction of the octadienyl side chain was indicated by the ^{1}H-NMR spectrum of **17a**, showing signals of olefinic protons at δ 5.42 (2H, m) and 5.47 (2H, m) and allylic protons at δ 2.67 (2H, H′-5), and by the fragment peak at *m/z* 296 ($M-H_2O$) in the MS. The epimer **17b** showed a similar ^{1}H-NMR spectrum to **17a**. Debenzylation with sodium in liquid ammonia[21] followed by Swern oxidation[18] gave *dl*-carbacerulenin (**5**) in 42% and 35% yields from **17a** and **17b**, respectively. The structure of **5** was elucidated on the basis of ^{1}H- and ^{13}C-NMR spectral data, MS data showing a peak at *m/z* 222 (M^+), and its infrared (IR) spectrum exhibiting a band at 1756 cm^{-1} (C=O). The stereochemistry at C-4 was assigned by analogy with the results obtained in the Grignard reaction of 2,3-epoxycyclopentanone below −40 °C, where the alkenyl lithium **16** attacks the carbonyl carbon from the opposite side of the epoxy group.[22,23] The fact that alkylation of both **15a** and **15b** led to the same product **5** supported this reaction mechanism. Thus, we concluded that *dl*-carbacerulenin **5** has the same relative configuration as that of the 5*S*-hydroxylactam **2a**.

FAS-Inhibitory Activity FAS-inhibitory activity of synthetic *dl*-carbacerulenin (**5**) was measured with yeast FAS. Purification of the FAS and measurement of the activity were carried out by the same procedures as described before.[8] The 50%-inhibitory concentration (IC_{50}) of **5** was 10.5 mM, while those of cerulenin (**1**) and the SH-inhibitor iodoacetamide (**19**) were 3.5 μM and 380 μM, respectively. The order of FAS-inhibitory activities observed for these compounds was not consistent with that of their second-order rate constants (*k*t*s*) in the reaction with the cysteine-SH group, which were determined to be 0.12, 0.12 and 0.41 $M^{-1}s^{-1}$ for **1**, **5**, and **19**, respectively. In order to

dissolve the samples, dimethylsulfoxide (DMSO) was used (5% final volume) and the rate was measured at 28 °C. The *kt* of the reaction between cysteine and **19** was previously reported to be 0.01 (0 °C).[24] The difference might be due to the difference of the reaction conditions.

These results may imply that the nitrogen functional group in cerulenin has an important role in the interaction of the compound with the enzyme.

Experimental

^{1}H-NMR spectra were measured on a JEOL GX-500 FT-NMR spectrometer (500 MHz for ^{1}H) and chemical shifts in $CDCl_3$ were recorded in δ relative to tetramethylsilane (TMS) as an internal standard. IR spectra were measured on a JASCO A-102 spectrometer. Low-resolution MS were measured under electron impact (EI) conditions at 70 eV and high-resolution (HR) MS were taken under fast atom bombardment (FAB) ionization on a JEOL JMS-HX110 mass spectrometer. Ultraviolet (UV) absorption spectra were recorded on a Shimadzu UV-300 spectrometer.

***trans*-2-Cyclopentene-1,4-diol (8),[12,15] *cis*-2-Cyclopentene-1,4-diol (9)[12-15] and Cyclopentene-3,4-diols (10 and 11)[15]** Cyclopentadiene (32.1 g, 486 mmol) was oxidized with 40% peracetic acid (45.6 g, 0.24 mmol) by the reported procedure.[10,11] Reaction products were separated by fractional distillation and, in order to determine the composition, a part of each distillate was separated by silica gel column chromatography ($CHCl_3$: MeOH = 20 : 1). On the basis of the reported ^{1}H-NMR spectral data,[12-14] it was proved that the first distillate (96—106 °C (3 mmHg), 4.8 g) consists of **8** : **9** : **10** : **11** in the ratio of 10 : 3 : 7 : 30 and the second distillate (110—114 °C (3 mmHg), 8.8 g), 57 : 29 : 11 : 3. **8**: ^{1}H-NMR ($CDCl_3$) δ: 2.09 (2H, t, J = 4.8 Hz, CH_2), 5.06 (2H, br t, J = 4.8 Hz, C<u>H</u>–OH), 6.02 (2H, d, J = 1.5 Hz, =CH). MS *m/z*: 100 (M^+), 99 (M − H), 82 (M − H_2O). **9**: ^{1}H-NMR ($CDCl_3$) δ: 1.54 (1H, ddd, J = 14.5, 3.8, 3.8 Hz, CH_2), 2.73 (1H, ddd, J = 14.5, 7.2, 7.2 Hz, CH_2), 4.68 (2H, m, C<u>H</u>–OH), 6.02 (2H, s, =CH). MS (a mixture of **9** and **10**) *m/z*: 100 (M^+), 82 (M − H_2O). **10**: ^{1}H-NMR ($CDCl_3$) δ: 2.24 (1H, br d, J = 17.3 Hz, CH_2), 2.80 (1H, br d, J = 17.3 Hz, CH_2), 4.24 (1H, m, C<u>H</u>OH), 4.63 (1H, m, =CHC<u>H</u>OH), 5.74 (1H, m, =CH), 5.88 (1H, m, =CH). **11**: ^{1}H-NMR ($CDCl_3$) δ: 2.36 (1H, br d, J = 17.5 Hz, CH_2), 2.62 (1H, br d, J = 17.5 Hz, CH_2), 4.33 (1H, m, C<u>H</u>OH), 4.60 (1H, m, =CHC<u>H</u>OH), 5.81 (1H, m, =CH), 5.95 (1H, m, =CH). Strong coupling was observed between the signals at δ 4.60 and 4.33 in the ^{1}H–^{1}H correlation spectrum. MS *m/z*: 100 (M^+), 99 (M − H), 82 (M − H_2O).

***trans*-4-Benzyloxy-2-cyclopentenol (12)[16] and *cis*-4-Benzyloxy-2-cyclopentenol (13)[16]** A 40% solution of peracetic acid (2.8 g, 15 mmol) was added with stirring to a solution of cyclopentadiene (2 g, 30 mmol) in CH_2Cl_2 (18 ml) containing sodium acetate (74 mg) and anhydrous Na_2CO_3 (3.8 g).[10,11] The temperature was maintained at 5—10 °C throughout the addition. The resulting mixture was stirred for 1 h at room temperature. The solid in the reaction was removed by filtration, and the filtered cake was washed with CH_2Cl_2. Benzyl alcohol (8.1 g, 75 mmol) and *p*-toluenesulfonic acid monohydrate (142 mg, 0.75 mmol) were added to the combined filtrate and washings. The resulting mixture was stirred for 1 h, followed by successive washing with 1 N HCl, saturated $NaHCO_3$ and brine, and drying over anhydrous magnesium sulfate. After evaporation of the solvent, the residue was chromatographed on silica gel (*n*-hexane : CH_2Cl_2 = 1 : 1) to give a mixture of **12** and **23** (870 mg, **12** : **13** = 2 : 1, 30% yield). **12**: ^{1}H-NMR ($CDCl_3$) δ: 2.00 (1H, ddd, J = 14.5, 6.8, 3.2 Hz, CH_2), 2.24 (1H, ddd, J = 14.5, 6.8, 3.2 Hz, CH_2), 4.51 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.54 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.82 (1H, m, CH–O), 5.05 (1H, m, CH–O), 6.08 (2H, m, =CH), 7.26—7.38 (5H, m, C_6H_5). MS (a mixture of **12** and **13**) *m/z*: 190 (M^+), 172 (M − H_2O), 107 ($C_6H_5CH_2O$), 91 ($C_6H_5CH_2$), 77 (C_6H_5). **13**: ^{1}H-NMR ($CDCl_3$) δ: 1.68 (1H, ddd, J = 14.0, 4.0, 4.0 Hz, CH_2), 2.67 (1H, ddd, J = 14.0, 7.0, 7.0 Hz, CH_2), 4.45 (1H, m, CH–O), 4.54 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.58 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.63 (1H, m, CH–O), 6.08 (2H, m, =CH), 7.26—7.38 (5H, m, C_6H_5).

4-Benzyloxy-2,3-epoxycyclopentanols (14a and 14b)[16] MCPBA (92.8 mg, 70% purity, 0.38 mmol) was added to a solution of the ethers **12** and **13** (50 mg, 0.26 mmol) in CH_2Cl_2 (1 ml), and the mixture was stirred overnight at room temperature. The resulting white solid was removed by filtration, and the filtrate was washed successively with 5% Na_2SO_3, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The reaction products were subjected to silica gel column chromatography (benzene : acetone = 10 : 1) to give **14a** (33 mg, 62% yield) and **14b** (5 mg, 9% yield). **14a**: ^{1}H-NMR ($CDCl_3$) δ: 1.43 (1H, m, CH_2), 2.18 (1H, dd, J = 13.7, 7.9 Hz, CH_2), 3.52 (1H, m, epoxy-O–CH), 3.57 (1H, epoxy-O–CH), 4.15 (1H, d, J = 5.8 Hz, CH–O), 4.51 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.53 (1H, m, CH–O), 4.59 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 7.28—7.38 (5H, m, C_6H_5). MS *m/z*: 206 (M^+), 91 ($C_6H_5CH_2$). **14b**: ^{1}H-NMR ($CDCl_3$) δ: 1.39 (1H, m, CH_2), 2.28 (1H, m, CH_2), 3.52 (1H, m, epoxy-O–CH), 3.57 (1H, m, epoxy-O–CH), 3.90 (1H, m, CH–O), 4.09 (1H, m CH–O), 4.61 (2H, ABq, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 7.28—7.38 (5H, m, C_6H_5). MS *m/z*: 206 (M^+), 91 ($C_6H_5CH_2$).

4-Benzyloxy-2,3-epoxycyclopentanones (15a and 15b) A solution of DMSO (1.29 g, 16.5 mmol) in CH_2Cl_2 (4 ml) was added dropwise to a solution of $(COCl)_2$ (1.06 g, 8.35 mmol) in CH_2Cl_2 (19 ml) cooled to −50 to −60 °C, and the mixture was stirred for 5 min. To this solution, a mixture of **14a** and **14b** (1.53 g, 7.4 mmol) in CH_2Cl_2 (7.5 ml) was added and, after 20 min of stirring, Et_3N (3.78 g) was added. The cooling bath was removed, the solution was brought to room temperature, H_2O (37.5 ml) was added, and the whole was extracted with CH_2Cl_2. The combined extract was washed successively with 1 N HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated.[18] Separation of the products by silica gel column chromatography (*n*-hexane : ether = 2 : 1) afforded **15a** (970 mg, 64% yield) and **15b** (337 mg, 22% yield). **15a**: ^{1}H-NMR ($CDCl_3$) δ: 2.16 (1H, d, J = 18.0 Hz, CH_2), 2.52 (1H, dd, J = 18.0, 5.8 Hz, CH_2), 3.38 (1H, d, J = 2.2 Hz, epoxy-O–CH), 3.92 (1H, d, J = 2.2 Hz, epoxy-O–CH), 4.38 (1H, d, J = 5.8 Hz, H-4), 4.54 (1H, d, J = 12.0 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.61 (1H, d, J = 12.0 Hz, C<u>H</u>$_2$C$_6$H$_5$), 7.28—7.37 (5H, m, C_6H_5). MS *m/z*: 204 (M^+), 91 ($C_6H_5CH_2$). **15b**: ^{1}H-NMR ($CDCl_3$) δ: 2.38 (1H, dd, J = 17.0, 8.0 Hz, CH_2), 2.45 (1H, dd, J = 17.0, 7.0 Hz, CH_2), 3.41 (1H, d, J = 2.3 Hz, H-2), 3.98 (1H, m, H-3), 4.19 (1H, ddd, J = 7.0, 7.0, 1.5 Hz, H-4), 4.65 (1H, d, J = 12.0 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.69 (1H, d, J = 12.0 Hz, C<u>H</u>$_2$C$_6$H$_5$), 7.30—7.40 (5H, m, C_6H_5). MS *m/z*: 204 (M^+), 91 ($C_6H_5CH_2$).

4-Benzyloxy-2,3-epoxy-1-((*E*,*E*)-3,6-octadienyl)cyclopentanols (17a and 17b) An *n*-pentane solution (1.4 M) of *tert*-butyl lithium (0.2 ml, 0.28 mmol) was added to a solution of (*E*,*E*)-3,6-octadienyl iodide[19] (50 mg, 0.21 mmol) in *n*-pentane (1 ml), cooled to −78 °C under an argon atmosphere, and the whole was stirred at −78 °C for 1 h. To this solution was added dropwise a solution of **15a** (53 mg, 0.26 mmol) in tetrahydrofuran (THF, 1 ml), and the resulting mixture was further stirred at −78 °C for 1.5 h. The reaction was quenched by addition of saturated aqueous Na_2SO_4 at −78 °C. The precipitated white solid was removed by filtration, and the filtrate was concentrated to afford an oily residue, which was purified by silica gel column chromatography (*n*-hexane : AcOEt = 10 : 1) to give **17a** (36 mg, 55% yield from the dienyl iodide). In the same manner, **15b** (220 mg) provided **17b** (61 mg, 18% yield). **17a**: ^{1}H-NMR ($CDCl_3$) δ: 1.55 (1H, dd, J = 14.5, 6.0 Hz, H-5), 1.66 (3H, br d, J = 4.5 Hz, CH_3), 1.74—1.88 (2H, m, H-1′), 1.99 (1H, d, J = 14.5 Hz, H-5), 2.23 (2H, m, H-2′), 2.67 (2H, br s, H-5′), 3.38 (1H, d, J = 2.5 Hz, epoxy-O–CH), 3.51 (1H, d, J = 2.5 Hz, epoxy-O–CH), 4.14 (1H, d, J = 6.0 Hz, H-4), 4.47 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 4.57 (1H, d, J = 11.5 Hz, C<u>H</u>$_2$C$_6$H$_5$), 5.42 (2H, m, =CH), 5.47 (2H, m, =CH), 7.30—7.40 (5H, m, C_6H_5). MS *m/z*: 296 (M − H_2O), 91 ($C_6H_5CH_2$). **17b**: ^{1}H-NMR ($CDCl_3$) δ: 1.43—1.62 (2H, m, CH_2), 1.66 (3H, d, J = 4.8 Hz, CH_3), 2.06—2.16 (4H, m, CH_2), 2.67 (2H, m, H-5′), 3.32 (1H, d, J = 2.8 Hz, H-2), 3.56 (1H, dd, J = 2.8, 1.2 Hz, H-3), 3.88 (1H, ddd, J = 7.8, 7.8, 1.2 Hz, H-4), 4.60 (2H, ABq, J = 11.7 Hz, C<u>H</u>$_2$C$_6$H$_5$), 5.37—5.48 (4H, m, =CH), 7.28—7.39 (5H, m, C_6H_5).

2,3-Epoxy-1-((*E*,*E*)-3,6-octadienyl)cyclopentane-1,4-diols (18a and 18b) An ether solution of **17a** (50 mg, 0.16 mmol) was added in portions to a solution of sodium (27 mg, 1.17 mmol) in liquid ammonia (2 ml) under a nitrogen atmosphere. The mixture was stirred for 5 min, then ammonium chloride was carefully added and ammonia was evaporated off. The reaction mixture was extracted with ether, and the extract was filtered through a short silica gel column. The filtrate was evaporated to give **18a** (34 mg, 95% yield). By the same procedure, **17b** (12 mg, 0.39 mmol) afforded **18b** (75 mg, 86% yield). **18a**: ^{1}H-NMR ($CDCl_3$) δ: 1.65 (3H, br d, J = 4.5 Hz, CH_3), 1.68 (1H, dd, J = 15.0, 6.0 Hz, H-5), 1.73—1.88 (2H, m, H-1′), 1.84 (1H, d, J = 15.0 Hz, H-5), 2.22 (2H, m, H-2′), 2.67 (2H, m, H-5′), 3.41 (1H, d, J = 2.2 Hz, epoxy-O–CH), 3.46 (1H, d, J = 2.2 Hz, epoxy-O–CH), 4.45 (1H, m, H-1), 5.42 (2H, m, =CH), 5.48 (2H, m, =CH). MS *m/z*: 224 (M^+), 206 (M − H_2O), 188 (M − 2 × H_2O). **18b**: ^{1}H-NMR ($CDCl_3$) δ: 1.30 (1H, dd, J = 13.0, 8.0 Hz, H-5), 1.50—1.64 (2H, m, H-1′), 1.65 (3H, br d, J = 4.5 Hz, CH_3), 2.15 (2H, m, H-2′), 2.19 (1H, dd, J = 13.0, 8.0 Hz, H-5), 2.67 (1H, m, H-5′), 3.38 (1H, d, J = 2.5 Hz, H-2), 3.56 (1H, dd, J = 2.5, 1.2 Hz, H-3), 4.10 (1H, m, H-1), 5.40—5.50 (4H, m, =CH). MS *m/z*: 224 (M^+), 206 (M − H_2O), 188 (M − 2 × H_2O).

2,3-Epoxy-4-hydroxy-4-((*E,E*)-3,6-octadienyl)cyclopentanone (*dl*-Carbacerulenin 5) Compounds **18a** (34 mg) and **18b** (68 mg) were oxidized by the same procedure as **14a** and **14b** to **15a** and **15b**. The reaction product was separated by silica gel column chromatography (*n*-hexane : ether = 2 : 1) to give **5** in 44% and 41% yields, respectively. **5**: ^{1}H-NMR ($CDCl_3$) δ: 1.66 (3H, br d, J = 4.8 Hz, CH_3), 1.70 (1H, m, H-1′), 1.77 (1H, m, H-1′), 2.14 (2H, m, H-2′), 2.29 (1H, d, J = 18.0 Hz, H-5), 2.40 (1H, d, J = 18.0 Hz, H-5), 2.66 (2H, br t, J = 5.5 Hz, H-5′), 3.49 (1H, d, J = 2.5 Hz, H-2), 3.79 (1H, d, J = 2.5 Hz, H-3), 5.40—5.50 (4H, m, =CH). ^{13}C-NMR (DMSO) δ: 17.62 (C-8′), 25.56 (C-2′), 34.90 (C-5′), 37.84 (C-1′), 42.71 (C-5), 56.19 (C-2), 62.65 (C-3), 72.85 (C-4), 125.0 (C-6′ or 7′), 128.5 (C-4′), 129.5 (C-6′ or 7′), 130.1 (C-3′), 206.8 (C-1). MS *m/z*: 222 (M^+). IR ($CHCl_3$) ν: 3590 (O–H), 2990 (C=C–H), 1756 (C=O) cm^{-1}. HRMS *m/z*: 223.1366, Calcd for $C_{13}H_{19}O_3$ (M+H): 223.1334.

Reaction of Cysteine with 1, 5 and 19[24] A DMSO solution of **1** (40 mM) was diluted with 50 mM potassium phosphate buffer (pH 6.5) (KPB) to prepare a 4 mM solution of **1**. Equivalent amounts of cysteine and **1** (initial concentration C_0 = 2 mM) were mixed and incubated at 28 °C. After 2, 5, 8, 13 and 18 min, samples (25 μl) were mixed with 975 μl of 5,5′-dithiobis(2-nitrobenzoic acid) solution (31.6 μg/ml in 200 mM KPB, pH 8). From the absorption at 405 nm, the residual cysteine concentration C_t was determined with the aid of a calibration curve obtained with a standard solution of cysteine. Reactions of **5** and **19** with cysteine were examined in the same way. The second-order rate constant was calculated using the equation $kt = 1/C_t - 1/C_0$.

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