Determination by Enantioselective Synthesis of the Absolute Configuration of CPE, a Potential Intermediate in Coronatine Biosynthesis

Tao Tao and Ronald J. Parry
Department of Chemistry MS60, Rice University, Houston TX 77251-1892, USA

Supporting Information

General Information.

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz. ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 100 MHz or on a Bruker Avance 500 spectrometer at 125 MHz. FT-IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. Optional rotations were recorded on a JASCO DIP-300 digital polarimeter at 589 nm. Mass spectra and high resolution mass spectra were obtained with a Finnigan MAT-95 spectrometer.

Analytical thin layer chromatography was performed on Whatman 0.25 mm silica gel 60-F plates. Flash chromatography was performed with 230-400 mesh silica gel. Normal phase preparative TLC was performed on Aldrich 1000 μm silica gel GF plates (20 x 20 cm). Normal phase analytical HPLC was performed on a Varian MICROSORB™ HPLC Si column (particle size: 5 μm, pore size 100 Å, 4.6 x 250 mm) with monitoring at 230 nm. Normal phase preparative HPLC was performed on a Varian DYNAMAX® Si HPLC column (Microsorb silica, particle size: 5 μm, pore size 100 Å, 10.0 x 250 mm) with 230 nm detection. Reverse phase preparative HPLC was performed on a Varian DYNAMAX C_{18} HPLC Column (Microsorb 100-5 C_{18} column, particle size: 5 μm, 10.0 x 250 mm) with monitoring at 230 nm.

Synthetic Procedures

1-Carbethoxy-5-hydroxycyclopent-1-ene (2): A solution of 2,5-dimethoxytetrahydrofuran (26 mL, 26.4 g, 0.2 mmol) in 0.6 N HCl (160 mL) was heated at 70 ^{o}C for 2 h. After cooling to room temperature, the solution was neutralized with saturated potassium bicarbonate. An aqueous solution of potassium carbonate (5M, 1 mL) was added, followed by triethyl phosphonoacetate (40 mL, 48 g, 0.2 mol), and an additional solution of potassium carbonate (5 M, 40 mL) while keeping the temperature below 30 ^{o}C. The reaction mixture was stirred at room temperature for 42 h, then extracted with ether (4 x 100 mL). The combined ether extracts were washed with saturated ammonium chloride twice, with water and brine, and dried over sodium sulfate. After solvent removal, the residue was purified by flash chromatography on silica gel to yield 15 g (46%) of product as a colorless oil. IR (neat) 3445, 2980, 1713, 1634, 1447, 1394, 1373, 1344, 1295, 1196, 1099, 1049, 983, 932, 762 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (m, 1H), 5.09 (m, 1H), 4.25 (q, 2H, *J* = 7.1 Hz), 2.80 (br.s, 1H), 2.60-2.70 (m, 1H), 2.30-2.50 (m, 2H), 1.82-1.91 (m, 1H), 1.32 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 145.5, 137.8, 74.2, 59.5, 31.6, 30.1, 13.4; MS (EI) *m/z* 83(M^+-COOEt), 128 (M^+-Et+1), 156 (M^+); HRMS (EI) calcd. for $C_8H_{12}O_3$ 156.0786, found 156.0780.

1-Carbethoxy-5-*tert*-butyldimethylsilyloxycyclopent-1-ene: To an iced-cold solution of 1-carbethoxy-5-hydroxycyclopent-1-ene (**2**) (7.6 g, 48.7 mmol) and imidazole (7.4 g, 109 mmol, 2.2 equiv.) in anhydrous DMF (45 mL) was added TBDMSCl (8.07 g, 53 mmol, 1.1 eq). The reaction mixture was allowed to warm to room temperature and monitored by TLC. After 4h, no starting material remained. The reaction mixture was poured into ice water (400 mL) and extracted with methylene chloride (4 x 100 mL). The combined methylene chloride extracts were washed with water followed by brine and dried over sodium sulfate. After filtration, the solvent

was removed, and the residue was purified by flash chromatography on silica gel to yield 11.43 g (87%) of the product as a colorless oil. IR (neat) 2955, 2930, 2857, 1721, 1292, 1254, 1099, 1068, 837, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (m, 1H), 5.05-5.07 (m, 1H), 4.17-4.24 (m, 2H), 2.63-2.64 (m, 1H), 2.30-2.36 (m, 1H), 2.10-2.22 (m, 1H), 1.82-1.86 (m, 1H), 1.30 (t, 3H, *J* = 7.2 Hz), 0.89 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 145.9, 139.0, 75.5, 59.9, 34.2, 30.7, 25.7 (3C), 18.0, 14.2, -4.94, -5.00; MS (CI, CH_4) *m/z* 139 (M^+-TBDMSO), 213 (M^+-C_4H_9), 255 (M^+-CH_3), 271 (M^++1); HRMS (CI, CH_4) calcd. for $C_{14}H_{27}O_3Si$ $(M+H)^+$ 271.1729, found 271.1729.

1-Hydroxymethyl-5-*tert*-butyldimethylsilyloxycyclopent-1-ene (3): To a solution of 1-carbethoxy-5-*tert*-butyldimethylsilyloxycyclopent-1-ene (5.4 g, 20 mmol) in dry methylene chloride (80 mL) chilled to –70 °C was added a 0 °C solution of DIBAL-H in toluene (1.0 M, 80.8 mL. 80.8 mmol, 2.02 eq). The reaction mixture was stirred at –70 °C for 5 h, then quenched by cautious addition of saturated ammonium chloride. The precipitate was removed by filtration and washed with methylene chloride. The filtrate was washed with brine once, and dried over sodium sulfate. After filtration, the solvent was removed and the residue was purified by flash chromatography on silica gel to yield 3.5 g (77%) of product as colorless oil. IR (neat) 3361, 2930, 2857, 1731, 1472, 1463, 1362, 1255, 1072, 1005, 939, 836, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.77 (m, 1H), 4.92 (m, 1H), 4.25 (d, 1H, *J* = 12.2 Hz), 4.23 (d, 1H, *J* = 12.2 Hz), 2.38-2.51 (m, 1H), 2.19-2.32 (m, 2H), 1.70-1.80 (m, 1H), 1.60 (br. s, 1H), 0.92 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.7, 128.9, 79.0, 60.5, 34.6, 29.7, 25.8, 18.0, -4.41, -4.99; MS (CI, CH_4) *m/z* 229 (M^++1); 213 (M^+-CH_3); HRMS (CI, CH_4) calc. for $C_{12}H_{25}O_2Si$ $(M+H)^+$ 229.1624, found 229.1626.

1-Iodomethyl-5-*tert*-butyldimethylsilyloxy-cyclopent-1-ene (4): To an ice-cold solution of 1-hydroxymethyl-5-*tert*-butyldimethylsilyloxycyclopent-1-ene (**3**) (1.37 g, 6 mmol) and triphenyl phosphine (4.72 g, 18 mmol, 3 eq) in anhydrous THF (60 mL) was added a solution of flame-dried zinc iodide (1.92 g, 6 mmol, 1 equiv.) in anhydrous THF (60 mL) via cannula. The mixture was stirred at 0 ^{o}C for 10 min and diethyldiazocarboxylate (2.78 mL, 3.08 g, 18 mmol, 3 eq) was then added dropwise via a syringe. The reaction mixture was stirred for 30 min, during which time a precipitate formed. The mixture was then poured onto a short silica column, and elution carried out with 10% ethyl acetate in hexane (v/v). Solvent was removed from the eluant, the residue extracted with 5 % ethyl acetate in hexanes, and the extract filtered. Solvent was removed from the filtrate, and the residue was purified by flash chromatography on silica gel to yield 1.16 – 1.40 g (57 - 69 %) of the product as a colorless to slightly yellowish oil. IR (neat) 2955, 2928, 2885, 2855, 1471, 1462, 1361, 1251, 1151, 1084, 1006, 953, 932, 883, 836, 777, 672 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (m, 1H), 4.96-4.98 (m, 1H), 4.06 (d, 1H, *J* = 7.3 Hz), 3.77 (d, 1H, *J* = 7.2 Hz), 3.75 (m, 1H), 2.27-2.36 (m, 2H), 2.07-2.15 (m, 1H), 1.82-1.89 (m, 1H), 1.67-1.76 (m, 1H), 0.90 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.7, 130.8, 76.4, 33.9, 30.1, 25.9, 18.0, 1.68, -4.26, -4.52; MS (CI. CH_4) *m/z* 155 (M^+-C_4H_9-I+1), 207 (M-TBDMSO)$^+$, 281 (M^+-C_4H_9), 323(M^+-CH_3), 339 (M^++1); HRMS (CI, CH_4) calcd. for $C_{12}H_{24}IOSi$ $(M+H)^+$ 339.0641, found 339.0642.

***(S)*-(-)-4-Benzyl-3-butyryl-2-oxazolidinone (6)** A flame-dried 250 mL three-neck flask was charged with anhydrous THF (50 mL), and then cooled in an dry-ice acetone bath. Triethylamine (2 mL, 1.45 g, 14.4 mmol, 1.2 eq), butyric acid (1.1 mL, 1.06 g, 12 mmol, 1.05 eq), and pivaloyl chloride (1.48 mL, 1.45 g, 12 mmol, 1.05 eq) were then added in sequence. The resulting slurry was stirred at –78 ^{o}C for 15 min, and at 0 ^{o}C for 45 min. At the same time, another flame-dried

flask was charged with (*S*)-(-)-4-benzyl-2-oxazolidinone (2 g, 11.3 mmol), and dry THF (50 mL). The resulting solution was cooled to –78 °C. Butyl lithium (1.6 M in hexane, 7.1 mL, 11.3 mmol, 1eq) was added via a syringe with stirring. The resulting yellowish solution was held at –78 °C until the next step. The reaction mixture containing the mixed anhydride produced from butyric acid and pivaloyl chloride was cooled to –78 °C again, and the lithium salt of (*S*)-(-)-4-benzyl-2-oxazolidinone was transferred dropwise into the anhydride-containing flask via a cannula. The resulted slurry was stirred at –78 °C for 15 min., warmed to 0 °C for 30 min., and then stirred at room temperature for an additional 30 min. The reaction was then cautiously quenched with saturated ammonium chloride (30 mL). The resulting mixture was concentrated in vacuo to remove the volatile components and the residue was then extracted with methylene chloride (3 x 50 mL). The combined extracts were washed with 1N NaOH (60 mL), followed by 1N $NaHSO_4$ (60 mL) and then dried over sodium sulfate. After filtration, the solvent was evaporated, and the residue was purified by flash chromatography on silica gel to yield 2.5 g (90%) of the product as sticky oil, which may solidify after long standing. $[\alpha]_D^{23}$ +58.7° (c, 1.07, $CHCl_3$); IR (neat) 3029, 2965, 2933, 2857, 1780, 1699, 1454, 1389, 1352, 1291, 1211, 1093, 703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.12-7.34 (m, 5H), 4.65-4.73 (m, 1H), 4.14-4.22 (m, 2H), 3.30 (dd, 1H, *J* = 13.4, 3.3 Hz), 2.80-3.00 (m, 2H), 2.77 (dd, 1H, *J* = 13.4, 9.6 Hz),1.70-1.80 (m, 1H) 1.01 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 153.4, 135.3, 129.3, 128.9, 127.2, 66.1, 55.0, 37.9, 37.3, 17.6, 13.6; MS(EI) *m/z* 77 ($C_6H_5^+$), 185 (M^+-CH_3-CO_2+1), 247 (M^+); HRMS (EI) calc. for $C_{14}H_{17}NO_3$ 247.1208, found 247.1203.

4-(*S*)-Benzyl-3-[2-(*R*)-(1-*tert*-butyldimethysilyloxy-2-cyclopenten-2-yl)-methyl]-butyryl-2-oxazolidinone (7): To a –78 °C solution of sodium bistrimethylsilylamide in THF (1.0 M, 6 mL, 6 mmol, 1.2 eq) was added a pre-cooled solution of (*S*)-4-benzyl-3-butyryl-2-oxazolidinone

(**6**)(1.28 g, 5 mmol) in THF (5 mL) via cannula. The resulting solution was stirred at –78 ^{o}C for an hour. Pre-cooled 1-iodomethyl-5-*tert*-butyldimethylsilyloxy-cyclopent-1-ene (**4**) (4.23 g, 12.5 mmol, 2.5 eq) was then added via cannula, and the resulting yellowish solution was stirred at –78 ^{o}C for 4 h. It was then quenched with saturated aqueous ammonium chloride. After removal of the volatile components in vacuo, the residue was extracted with methylene chloride (3 x 20 mL), and the combined extracts were washed with brine and dried over sodium sulfate. After filtration, the solvent was removed in vacuo to give a residue which was purified by flash chromatography on silica gel to yield 2.2 g (94%) of the product as a sticky liquid, which solidified on long standing. $[\alpha]_D^{23}$ +34.8^{o} (c, 0.935, $CHCl_3$); IR (neat) 2957, 2929, 2856, 1782, 1698, 1389, 1349, 1249, 1211, 1195, 1096, 836, 775 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-7.37 (m, 5H), 5.57 (m, 1H), 4.68-4.76 (m, 2H), 4.14-4.21 (m, 2H), 4.08-4.14 (m, 1H), 3.28 (dd, 1H, *J* = 10.6, 2.6 Hz), 2.68 (dd, 1H, *J* = 10.8, 8.0 Hz), 2.41-2.50 (m, 1H), 2.36-2.47 (m, 2H), 2.17-2.28 (m, 2H), 1.60-1.78 (m, 3H), 0.96 (t, 3H, *J* = 3.0 Hz), 0.94 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 153.1, 144.4, 135.4, 129.4 (2C), 128.9 (2C), 127.3, 126.7, 79.7, 65.8, 55.5, 42.4, 37.9, 34.1, 29.8, 29.7, 29.3, 25.9 (3C), 25.6, 11.2, -4.31, -4.74; MS *m/z* 400 (M^{+}-C_4H_9), 457 (M^{+}); HRMS (EI) calcd. for $C_{26}H_{39}NO_4Si$ 457.2648, found 457.2655.

Methyl 2-(*R*)-[(1-*tert*-butyldimethysiloxy-2-cyclopenten-2-yl)-methyl]-butyrate (8): To an ice-cold solution of 4-(*S*)-benzyl-3-[2-(*R*)-(1-*tert*-butyldimethysiloxy-2-cyclopenten-2-yl)-methyl]-butyryl-2-oxazolidinone (**7**) (1.5 g, 3.25 mmol) in THF:water (3 : 1, 64 mL) 30% hydrogen peroxide (1.56 mL, 26 mmol, 8 eq) was added, followed by $LiOH{\bullet}H_2O$ (270 mg, 6.5 mmol, 2 eq). The resulting solution was stirred at 0 ^{o}C for 3 hours, then an aqueous solution of sodium sulfite (1.5 N, 19.4 mL, 29 mmol, 9 eq) was added, and the mixture was stirred for an additional 30 min. The volatile solvents were removed in vacuo and the aqueous solution was

extracted twice with methylene chloride. The aqueous layer was acidified to pH 2 and extracted twice with ethyl acetate. The combined ethyl acetate extracts were washed with brine once, dried over sodium sulfate, and concentrated in vacuo to give a residue that was dried briefly *in vacuo* to yield about 820 mg of the crude acid. The crude acid was dissolved in dry methanol (5.5 mL), the solution cooled to 0^{o}C, and DCC (684 mg, 3.9 mmol, 1.2 eq) and DMAP (68 mg) were then added with stirring. The mixture was allowed to reach room temperature and then stirred for 4 h. At the end of this time, the reaction mixture was filtered to remove the precipitate. The filtrate was concentrated in vacuo and the residue purified by flash chromatography on silica gel to yield 708 mg, (70 %, two steps) of the desired ester as colorless liquid. Alternately, the crude acid was dissolved in methanol (50 mL), and treated with excess CH_2N_2. After removal of the solvents in vacuo, the residue was purified by flash column chromatography on silica gel to give the ester in comparable yield. Spectral data for the crude acid were: $[\alpha]_D^{23}$ –7.33^{o}, (c, 1.19, $CHCl_3$); IR (neat) 3418, 2958, 2858, 1711, 1472, 1463, 1362, 1254, 1178, 1093. 1006, 837, 777, 669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9-12 (br. s, 1H), 5.61 (m, 1H), 4.72-4.75 (m, 1H), 2.55-2.69 (m, 1H), 2.35-2.45 (m, 2H), 2.10-2.35 (m, 3H), 1.60-1.70 (m, 2H), 1.48-1.60 (m, 1H), 0.93 (t, 3H, *J* = 8 Hz), 0.92 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0, 143.4, 127.8, 79.5, 45.5, 34.1, 30.3, 29.8, 25.9 (3), 25.4, 18.1, 11.7, -4.38, -4.82; MS (CI, CH_4) *m/z* 133 (TBDMSOH+H)$^{+}$, 165 (M-TBDMS-H_2O)$^{+}$, 183 (M-TBDMS)$^{+}$, 281 (M-OH)$^{+}$, 297 (M-H)$^{+}$. 299 (M+H)$^{+}$; HRMS (CI, CH_4) calcd. for $C_{16}H_{31}O_3Si$ (M+H)$^{+}$ 299.2042, found 299.2041.

Spectral data for the purified ester were: $[\alpha]_D^{23}$ –7.33^{o} (c, 1.19, $CHCl_3$); IR (neat) 2958, 2857, 1739, 1472, 1462, 1435, 1361, 1257, 1200, 1163, 1091, 885, 837, 775 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.48-5.49 (m, 1H), 4.66-4.68 (m, 1H), 3.67 (s, 3H), 2.55-2.59 (m, 1H), 2.35-2.40 (m,

2H), 2.10-2.30 (m, 3H), 1.50-1.70 (m, 3H), 0.91 (s, 9H), 0.90 (t, 3H, *J* = 8 Hz), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 143.9, 126.8, 79.2, 51.2, 45.2, 34.0, 30.2, 29.7, 25.8 (3), 25.5, 18.1, 11.6, -4.42, -4.90; MS (EI) *m/z* 223 (M^+-C_4H_9-OCH_3-1), 311 $(M-1)^+$, 312 (M^+); HRMS (EI) calc. for $C_{17}H_{32}O_3Si$ 312.2121, found 312. 2118.

Methyl 2-(*R*)-[(1-hydroxy-2-cyclopenten-2-yl)-methyl]-butyrate (9): A dry plastic bottle was charged with a solution of methyl 2-(*R*)-[(1-*tert*-butyldimethysiloxy-2-cyclopenten-2-yl)-methyl]-butyrate (125 mg, 0.4 mmol) in THF (8 mL), and then cooled in an ice-water bath. To this solution was added HF-pyridine (90.5 mL) via plastic syringe. The resulting solution was stirred at 0 ^{o}C for 4 h, then diluted with diethyl ether (60 mL). Saturated sodium bicarbonate was added until no additional carbon dioxide was evolved. After separation, the aqueous layer was extracted with an additional volume of ether (20 mL), the combined ether extracts were washed once with brine, and they were then dried over sodium sulfate. After solvent removal, the residue was purified by flash chromatography on silica gel to yield 50 mg (70%) of the product as a colorless liquid. $[\alpha]_D^{23}$ –46.5^o (c, 0.17, $CHCl_3$); IR (neat) 3418, 2963, 2855, 1737, 1436, 1204, 1166, 1047 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56-5.57 (m, 1H), 4.58-4.59 (m, 1H), 3.68 (s, 3H), 2.55-2.65 (m, 1H), 2.43-2.53 (m, 1H), 2.21-2.43 (m, 3H), 2.12-2.21 (m, 1H), 1.82 (d, 1H, *J* = 7.4 Hz), 1.50-1.72 (m, 3H), 0.91 (t, 3H, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 143.6, 129.1, 78.7, 51.5, 46.0, 33.8, 30.7, 29.6, 25.6, 11.6; MS (CI, CH_4) *m/z* 181 (M^+-H_2O+1), 199 (M^++1); HRMS (CI, CH_4) calcd. for $C_{11}H_{19}O_3$ $(M+H)^+$ 199.1334, found 199.1328.

Methyl (*R*)-2-[(1-oxo-2-cyclopenten-2-yl)-methyl]-butyrate (10): To a solution of methyl 2-(*R*)-[(1-hydroxy-2-cyclopenten-2-yl)-methyl]-butyrate (200 mg, 1 mmol) in dry benzene (12 mL) was added 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (250 mg, 1.1 mL, 1.1 eq). The resulting orange solution was stirred at room temperature overnight, during which time an orange

precipitate was formed. After filtration, the filtrate was concentrated in vacuo, and the residue was purified by flash chromatography to yield 170 mg (88%) of the product as a colorless liquid. $[\alpha]_D^{23}$ –32.9° (c, 0.17, $CHCl_3$); IR (neat) 2964, 2878, 1734, 1699, 1460, 1437, 1345, 1268, 1207, 1165, 1003 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32-7.35 (m, 1H), 3.65 (s, 3H), 2.48-2.62 (m, 4H), 2.35-2.40 (m, 3H), 1.50-1.57 (m, 2H), 0.91 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.3, 175.7, 158.8, 143.5, 51.3, 45.3, 34.2, 27.0, 26.4, 25.4, 11.4; MS (EI) *m/z* 136 (M^+-COOMe+1), 164 (M^+-OCH_3+1), 196 (M^+); HRMS (EI) calcd. for $C_{11}H_{16}O_3$ 196.1099, found 196.1097.

Fermentation of Pseudomonas Syringae. Isolation and Purification of CPE Methyl Ester.

Media preparation

(a) King's B medium: Difco proteose peptone #3 (0.4 g), glycerol (0.22 g), KH_2PO_4 (32 mg), and $MgSO_4$•$7H_2O$ (32 mg) were dissolved in water (20 mL), and the resulting solution was then autoclaved.

(b) Seed medium: KH_2PO_4 (0.41 g), K_2HPO_4 (0.36 g), $MgSO_4$•$7H_2O$ (20 mg), α-(+)-D-glucose (0.5 g), and Difco yeast extract (0.5 g) were dissolved in water (80 mL), and the pH was adjusted from 6.6 to 6.8 with 1 N NaOH. The total volume was then brought to 100 mL, and solution was autoclaved.

(c) Production medium: KH_2PO_4 (8.2 g), K_2HPO_4 (7.2 g), and $MgSO_4$•$7H_2O$ (400 mg) were dissolved in water (1.8 L) and the pH was adjusted to 6.8 with 1 N NaOH. The total volume then was brought to 2 L, distributed equally into 5 2L Erlenmeyer flasks and autoclaved. α-(+)-D-glucose (20 g), NH_4Cl (2 g), and (D)-(+)-biotin (2 mg) were dissolved in water to give a final

volume of 120 mL. Before inoculation, an aliquot (24 mL) of this solution was added to each of the autoclaved 2-liter flasks through a sterile filter.

Fermentation to produce CPE (2 L scale): In a laminar flow hood, autoclaved King's B medium (5 mL) was transfer to a 50-mL sterile centrifuge tube, and 100 µl of thawed *P. syringae* PG4180 glycerol stock was added. After incubation at 30 ^{o}C and 280 RPM for 24 hours, this culture was transfer to the autoclaved seed medium (100 mL) and incubated under the same conditions for another 24 to 28 hours. The seed culture (100 ml) was then distributed between 5 2-L Erlenmeyer flasks which each contained 400 mL of complete production medium and the cultures were incubated at 18 ^{o}C, and 250 RPM. The production of CPE was monitored by normal phase HPLC (4.5 x 250 mm Si column; isocratic elution with mobile phase consisting of isopropanol:hexane, 8:92 v/v; the flow rate was 1.0 ml/min, and the retention time for synthetic methyl (*R*)-CPE was ca. 9 min). For analysis, 20 mL of the fermentation broth was centrifuged, and the supernatant was acidified to pH ~2 with 2.5 M H_2SO_4 (~ 4 drops). The acidified broth was extracted with ethyl acetate (3 x 10 mL) and the extracts dried over Na_2SO_4. The residue obtained by removal of the ethyl acetate from the dried extracts was dissolved in methanol (15 mL), and treated with excess diazomethane, prepared from Diazald (1.17 g). The solvent was then removed and the residue dissolved in isopropanol:hexane (3 : 7 v/v, 100 µL) for HPLC analysis. Maximum CPE production occurred after approximately 3 days. The scale of the fermentation could be increased to 8 L without affecting the timing or level of CPE production.

Isolation and purification of CPE methyl ester: After 3 days, cells were removed by centrifugation and the combined supernatants were acidified to pH 2.5 with 2.5 M H_2SO_4. The acidified solution was extracted with ethyl acetate (3 x 600 mL, for 2 L production), and the combined extracts were washed with brine once and dried over Na_2SO_4. After removal of the

solvent, the residue was dissolved in methanol (50 mL, for 2 L production) and treated with diazomethane prepared from Diazald (23.4 g). After solvent removal, the residue was dissolved in isopropanol:hexane (3:7), and purification carried out by preparative TLC (Aldrich silica gel GF_{254}, 20 X 20 cm, 1000 μm, developed with 8% isopropanol in hexane). After two developments, the band corresponding to CPE methyl ester (R_f 0.35) was removed from the TLC plates and extracted with 30% isopropanol in hexane. After removal of the solvent *in vacuo*, the residue was dissolved in 8% isopropanol in hexane (~ 200 μL), and purified by normal phase preparative HPLC (10 mm x 250 mm Si column; flow rate 4.0 mL/min; isocratic elution with mobile phase consisting of 8:92 isopropanol:hexane; retention time was ca. 9 min). From a total of 24 L of fermentation broth processed in this way, about 13 mg of partially purified CPE methyl ester was obtained. This was dissolved in methanol:water (7:3, v/v, 500 μL), and purified by reverse phase preparative HPLC (10 mm x 250 mm C_{18} column; flow rate; 2.5 mL/ min; isocratic elution with methanol:water, 7:3; retention time ca. 8.6 min) to yield 3 mg of CPE methyl ester which displayed chromatographic and spectral properties identical to the synthetic compound . The purified CPE methyl ester exhibited $[\alpha]_D^{23}$ -33.0° (c 0.12, $CHCl_3$).