Chemoenzymatic Synthesis of the Microbial Elicitor (−)-Syringolide via a Fructose 1,6-Diphosphate Aldolase-Catalyzed Condensation Reaction

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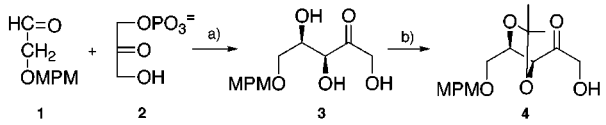
Syringolide 2, an elicitor of the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* which triggers a hypersensitive defense response in resistant soybean plants, has been synthesized in five steps via a fructose 1,6-diphosphate aldolase reaction.

Introduction

Some plant pathogens produce signal molecules (elicitors) which are recognized specifically by resistant plants and enable the plants to initiate active defense responses against these pathogens.[1] In 1993, Sims et al.[2] isolated novel nonprotein elicitors syringolides 1 and 2 from *Pseudomonas syringae* pv. *tomato*. These compounds elicit a hypersensitive response in resistant cultivars of soybeans. This defense reaction involves a rapid, localized cell death and accumulation of phytoalexins (antimicrobial compounds) around the infection site. Syringolides attract considerable interest since they have features in common with antigens that are recognized by the immune systems of vertebrates. Recently, several enantioselective synthesis of syringolides have been reported.[3] Syringolides have been synthesized from chiral pool precursors such as xylose,[3a−c] tartaric acid,[3d,e] glyceraldehyde,[3f] or via the Sharpless catalytic asymmetric dihydroxylation of butenolides.[3g,h]

The use of aldolases in the synthesis of carbohydrates and close analogues (azasugars, cyclitols) has been amply demonstrated.[4] However, the application of aldolases for the synthesis of natural products other than sugars has received very little attention. The syntheses of brevicomin,[5a] aspicilin[5b] (C3−C9 fragment), pentamycin[5c] (C11−C16 fragment), and amphotericin[5d] (C12−C20 fragment) are rare examples of this strategy. We report here a chemoenzymatic synthesis of (−)-syringolide 2 via a fructose 1,6-diphosphate aldolase (FDP aldolase) reaction.

Scheme 1[a]



[a] Reagents and conditions: (a) i) FDP aldolase, H_2O/DMF, ii) acid phosphatase; 65%; (b) acetone, p-TsOH, 67%

Results and Discussion

Aldehyde **1**[6] was first prepared in two steps by alkylation of allyl alcohol with *p*-methoxyphenylmethyl chloride and ozonolysis of the corresponding ether, followed by reductive workup with dimethyl sulfide.

The reaction of aldehyde **1** with dihydroxyacetone phosphate **2** (DHAP)[7] in the presence of fructose 1,6-diphosphate aldolase in water/DMF (10/1), followed by hydrolysis in situ of the intermediate phosphate ester with acid phosphatase, afforded ketotriol **3** in 65% yield (Scheme 1). FDP aldolase catalyzes the formation of C−C bonds having the D-threo (3*S*,4*R*) configuration;[8] compound **3** is presumed to have the 3*S*,4*R* absolute configuration. Compound **3** was treated with acetone under acid catalysis to give acetonide **4**. Acylation of **4** with octanoyl Meldrum's derivative[9] **5** in refluxing THF led to the corresponding ester which upon standing or treatment with silica gel gave keto-ester **6** via an intramolecular Knoevenagel reaction (Scheme 2). Removal of the MPM protecting group was accomplished with

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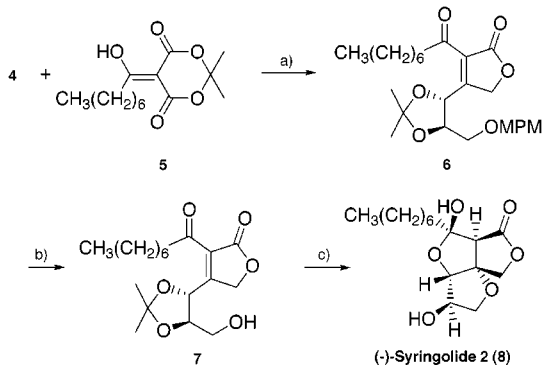
Scheme 2[a]



[a] Reagents and conditions: (a) i) THF, reflux, ii) SiO_2, hexane/EtOAc, 9/1; 71%; (b) DDQ, CH_2Cl_2-H_2O, 83%; (c) TsOH, H_2O-acetone, 55%

DDQ. Treatment of the resultant compound **7** with an excess of *p*-toluenesulfonic acid in acetone−water provided syringolide 2 (**8**) in 77%. Recrystallization from pentane/ether (2/1) gave analytically pure syringolide 2 in 54% yield, [[α]$^{25}_D$ −75.0 (*c* 0.06, $CHCl_3$); lit.[2] [α]$^{24}_D$ −75.91 (*c* 0.22, $CHCl_3$)]. The spectroscopic and physical data of synthetic syringolide 2 (**8**) were identical to those reported in the literature.[2,3] The synthesis of the natural enantiomer of syringolide 2 proved that the aldolase-catalyzed reaction provided an addition product (**3**) with the 3*S*,4*R* configuration.

In conclusion, we have completed the enantioselective synthesis of the natural enantiomer of syringolide 2 in five steps and 14% overall yield via an aldolase-catalyzed condensation. The present work demonstrates that the aldolase-catalyzed reactions are efficient processes in the asymmetric synthesis of natural products.

Experimental Section

General. Aldolase (D-fructose-1,6-bisphosphate-D-glyceraldehyde-3-phosphate-lyase; EC 4.1.2.13, from rabbit muscle) and acid phosphatase (from wheat germ, EC 3.1.3.2) were purchased from Sigma Chem. Co. Melting points are uncorrected. NMR spectra were recorded at 300 MHz (^{1}H) and 75.44 MHz (^{13}C).

***p*-Methoxybenzyloxyacetaldehyde (1).** A solution of allyl alcohol (0.340 mL, 5 mmol) in THF (100 mL) was added dropwise to a suspension of NaH (180 mg, 7.5 mmol) in THF (24 mL) at 0 °C under dry atmosphere. The reaction mixture was stirred at 0 °C for 45 min. Tetrabutylammonium iodide (25 mg, 0.067 mmol) and *p*-methoxybenzyl chloride (0.88 mL, 6.5 mmol) were added, and reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 12 h. The reaction mixture was filtered, and the solvent was evaporated. The residue was dissolved in ether, and the organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (petroleum ether /diethyl ether, 9/1) to give allyl *p*-methoxybenzyl ether[6b] (850 mg, 95%) as a colorless oil. IR (neat) 3100−3000, 1650, 1300−1100 cm^{-1}; ^{1}H NMR ($CDCl_3$) 7.27 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.87 (m, 1H), 5.31 (ddd, J_1 = 16.1 Hz, J_2 = 3.2 Hz, J_3 = 1.4 Hz, 1H), 5.22 (ddd, J_1 = 10.0 Hz, J_2 = 2.5 Hz, J_3 = 1.4 Hz, 1H), 4.46 (s, 2H), 4.05 (m, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$) 159.10, 134.79, 130.30, 129.23, 116.86, 113.67, 71.67, 70.75, 55.12; MS (E1, 70 eV) 178 (M$^+$). A solution of allyl *p*-methoxybenzyl ether (800 mg, 4.49 mmol) in CH_2Cl_2 (30 mL) was cooled to − 78 °C, and a stream of O_2/O_3 was passed through until the persistence of a blue color. Nitrogen was bubbled through the solution to remove the excess ozone. Dimethyl sulfide (496 mg, 6.75 mmol) was added dropwise, and the temperature was raised to room temperature. The solution was stirred at room temperature for 3 h, and the solvent was evaporated. The crude product was purified by flash chromatography (hexane/EtOAc, 9/1) to give aldehyde **1**[6](647 mg, 3.59 mmol) as a colorless oil. IR (neat) 3100−3090, 1760, 1300−1100 cm^{-1}; ^{1}H NMR ($CDCl_3$) 9.68 (s, 1H), 7.28 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.55 (s, 2H), 4.05 (s, 2H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$) 200.48, 159.52, 129.62, 128.77, 113.87, 74.89, 73.20, 55.15; HRMS (EI, 70 eV) calcd for $C_{10}H_{12}O_3$ (M$^+$) 180.0786, found 180.0792 ± 0.0005.

(3*S*,4*R*)-5-*p*-Methoxybenzyloxy-1,3,4-trihydroxy-2-pentanone (3). Aldehyde **1** (600 mg, 3.33 mmol) was added to a solution of DHAP **2** (680 mg, 4.0 mmol) in 25 mL of water/DMF (9/1) under nitrogen atmosphere, and the pH was adjusted to 6.8 with 1 N NaOH. Aldolase was added (36 mg, 360 U), and the mixture was shaken at room temperature. After 24 h and again after 48 h, the pH was readjusted to 6.8, and additional aldolase (33 mg, 330 U) and DHAP (374 mg, 2.2 mmol) were added. After 72 h, the pH was set at 4.8 with 1 N HCl, the acid phosphatase (143 mg, 57 U) was added, and the mixture was stirred at room temperature for 12 h. Additional phosphatase (57 U) was added, and the mixture was stirred for 12 h. The pH was then raised to 7 with 1 N NaOH. The solution was freeze-dried, the residue was taken up in ethyl acetate, and the solution was filtered and evaporated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 19/1) to afford **3** as an oil (588 mg, 2.18 mmol, 65%). [α]$^{25}_D$ −1.8 (*c* 2.0, $CHCl_3$); IR (neat) 3640−3000, 1730, 1300−1100 cm^{-1}; ^{1}H NMR ($CDCl_3$) 7.24 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 4.57 (d, J = 19.8 Hz, 1H), 4.47 (s, 2H), 4.42 (d, J = 19.8 Hz, 1H), 4.33 (d, J = 2.3 Hz, 1H), 4.12 (m, 1H), 3.81 (s, 3H), 3.64 (dd, J_1 = 4.4 Hz, J_2 = 1.2 Hz, 2H), 2.2 (br s, 3H); ^{13}C NMR ($CDCl_3$) 211.05, 159.83, 129.46, 129.23, 113.84, 76.12, 73.22, 70.58, 66.65, 55.15; HRMS (CI, NH_3) calcd for $C_{13}H_{18}O_6$ (M$^+$) 270.1103, found 270.1108 ± 0.0008.

(3*S*,4*R*)-5-*p*-Methoxybenzyloxy-3,4-isopropylidenedioxy-1-hydroxy-2-pentanone (4). A solution of **3** (541 mg, 2.0 mmol) and *p*-toluenesulfonic acid (45 mg) in freshly distilled acetone (10 mL) was stirred for 5 h in the presence of molecular sieves (4 Å, 40 mg). The mixture was filtered, neutralized with triethylamine (2 mL), and concentrated. The residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried and evaporated. The crude product was purified by flash chromatography (ether/hexane, 2/3) to give **4** (416 mg, 1.34 mmol, 67%) as a colorless oil. [α]$^{25}_D$ −7.7 (*c* 1.45, $CHCl_3$); IR (neat) 3600−3100, 1730, 1380, 1370 cm^{-1}; ^{1}H NMR ($CDCl_3$) 7.18 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 4.47 (s, 2H), 4.44 (m, 2H), 4.31 (d, J = 8.0 Hz, 1H), 4.11 (m, 1H), 3.72 (s, 3H), 3.67 (dd, J_1 = 10.7 Hz, J_2 = 3.1 Hz, 1H), 3.55 (dd, J_1 = 10.7 Hz, J_2 = 5.2 Hz, 1H), 2.88 (t, J = 5.0 Hz, 1H), 1.39 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$) 209.10, 159.21, 129.67, 129.22, 113.72, 111.36, 79.98, 76.90, 73.21, 69.23, 66.12, 55.13, 26.65, 26.01; HRMS (CI, NH_3) calcd for $C_{16}H_{22}O_6$ (M$^+$) 310.1416, found 310.1421 ± 0.0009.

(1′*R*,2′*R*)-3-[1′,2′-(Isopropylidenedioxy)-3′-(*p*-methoxybenzyloxy)propyl]-2-octanoyl-4-olide (6). A solution of **4** (190 mg, 0.612 mmol) and octanoyl Meldrum's derivative **5** (prepared by stirring equimolar amounts of 2,2-dimethyl-1,3-dioxane-4,6-dione and *n*-octanoyl chloride in CH_2Cl_2 at 0 °C for 8 h) in THF was stirred at reflux temperature for 3 h. The solvent was evaporated, and the residue was dissolved in hexanes−ethyl acetate 9/1 (15 mL) in the presence of silica gel (1 g). The mixture was stirred at room temperature for 18 h. Silica gel was filtered, and the solvents were evaporated. Flash chromatography (EtOAc/hexane, 3/7) gave **6** (202 mg, 71%) as a colorless oil. [α]$^{25}_D$ −23.6 (*c* 1.2, $CHCl_3$); IR (neat) 1780, 1700, 1640, 1370, 1360 cm^{-1}; ^{1}H NMR ($CDCl_3$) 7.23 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 5.43 (d, J = 8.2 Hz, 1H), 5.03 (d, J = 19.8 Hz, 1H), 4.83 (d, J = 19.8 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 4.45 (d, J = 11.6 Hz, 1H), 3.97 (m, 1H), 3.78 (s, 3H), 3.72 (d, J = 4.8 Hz, 2H), 2.91 (t, J = 7.0 Hz, 2H), 1.57 (m, 2H), 1.43 (s, 6H), 1.27 (m, 8H), 0.86 (t, J = 5.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) 196.56, 172.47, 170.08, 159.16, 129.69, 129.31, 126.71, 113.61, 110.96, 80.69, 73.76, 73.15, 69.78, 68.88, 55.10, 41.84, 31.53, 28.95, 28.87, 26.64, 23.03, 22.45, 13.92; HRMS (CI, isobutane) calcd for $C_{26}H_{36}O_7$ (M$^+$) 460.2461, found 460.2464 ± 0.0014.

(1′*R*,2′*R*)-3-[3′-(Hydroxy)-1′,2′-(isopropylidenedioxy)-propyl]-2-octanoyl-2-buten-4-olide (7). Compound **6** (175 mg, 0.38 mmol) was dissolved in a mixture of CH_2Cl_2 (10 mL) and water (0.5 mL). DDQ (130 mg, 1.5 equiv) was added, and the mixture was stirred at room temperature. The reaction was monitored by TLC and quenched after 3 h. The reaction mixture was diluted with CH_2Cl_2, filtered, and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and evaporated, and the crude product was purified by flash chromatography (EtOAc/hexane, gradient 1/4 to 2/3) to yield **7** (107 mg, 83%) as a colorless oil. $[\alpha]^{25}_D$ −60.0 (*c* 1.6, $CHCl_3$); IR (neat) 3600−3100, 1790, 1710, 1620, 1370, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) 5.39 (d, J = 8.0 Hz, 1H), 5.10 (d, J = 19.8 Hz, 1H), 4.85 (d, J = 19.8 Hz, 1H), 3.96−3.80 (m, 3H), 3.09 (m, 1H), 2.92 (m, 1H), 1.58 (m, 2H), 1.45 (s, 6H), 1.27 (m, 8H), 0.97 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 198.75, 173.33, 169.74, 126.75, 110.54, 81.44, 73.82, 69.02, 61.42, 41.92, 31.49, 28.91, 28.84, 26.65, 23.04, 22.46, 13.93; HRMS (CI, NH_3) calcd for $C_{18}H_{29}O_6$ (MH^+) 341.1964, found 341.1956 ± 0.0010.

(−)-Syringolide 2 (8). A solution of **7** (70 mg, 0.21 mmol) and 10 equiv of TsOH (399 mg, 2.1 mmol) in water/acetone (1.2/1, 10 mL) was stirred for 56 h at room temperature. The solution was neutralized with saturated aqueous $NaHCO_3$ (2 mL), and the solvents were concentrated in vacuo. The residue was taken up in EtOAc (20 mL) and washed with brine (3 × 5 mL). The organic layer was dried (Na_2SO_4) and evaporated. The solid product was redissolved in EtOAc (3 mL), and the solution was filtered on silica gel (2 g). The solution was evaporated, and the solid residue was washed with hexane to give syringolide 2 (49 mg, 77%) as a pale yellow solid. Recrystallization from pentane/ether (2/1) gave pure syringolide **2** (34 mg, 54%) as a colorless solid. Mp 121−123 °C; $[\alpha]^{25}_D$ −75.0 (*c* 0.06, $CHCl_3$); lit.[2] mp 123−124 °C, $[\alpha]^{24}_D$ −75.91 (*c* 0.22, $CHCl_3$); lit.[3a] mp 120−122 °C, $[\alpha]^{20}_D$ −74.7 (*c* 0.10, $CHCl_3$); IR (KBr) 3600−3100, 1700 cm^{-1}; ^1H NMR (CD_3COCD_3) 5.38 (d, J = 1.9 Hz, 1H), 4.68 (d, J = 10.2 Hz, 1H), 4.49 (br s, 1H), 4.33 (d, J = 10.2 Hz, 1H), 4.30 (d, J = 4.2 Hz, 1H), 4.13 (m, 1H), 3.94 (dd, J_1 = 10.1 Hz, J_2 = 1.0 Hz, 1H), 3.83 (dd, J_1 = 10.1 Hz, J_2 = 2.8 Hz, 1H), 3.10 (s, 1H), 1.89 (m, 2H), 1.70−1.40 (m, 2H), 1.30 (m, 8H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 172.70, 108.72, 99.00, 92.16, 75.50, 75.32, 74.81, 59.63, 39.36, 32.40, 30.45, 29.40, 24.27, 23.15, 14.18. HRMS (CI, NH_3) calcd for $C_{15}H_{25}O_6$ (MH^+) 301.1651, found 301.1657 ± 0.0009.

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Supporting Information Available: Spectrometric information (^1H and ^{13}C NMR) for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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