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Synthesis of (*R*)- and (*S*)-10,16-dihydroxyhexadecanoic acid: cutin stereochemistry and fungal activation

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Abstract

The first asymmetric syntheses of the cutin monomers (R)- and (S)-10,16-dihydroxyhexadecanoic acid (10,16-DHPA) and confirmation of (S)(+)-absolute configuration for 10,16-DHPA derived from tomato are reported. The individual DHPA stereoisomers display differences in their ability to activate the fungal pathogen *Colletotrichum trifolii*.

Keywords: Tomato; *Lycopersicon esculentum*; Solonaceae; Cutin monomers; DHPA; *Colletotrichum trifolii*; Dihydroxyhexadecanoic acid; Asymmetric synthesis

1. Introduction

Cutin, the outer coating on all aerial surfaces of plants, is a polyester primarily composed of polyhydroxylated fatty acids (Kolattukudy, 2001). Upon attack by some pathogenic fungi, the cutin layer is cleaved by cutinolytic enzymes, and the presence of cutin monomers released has been found to induce expression of the fungal gene coding for these same cutinases (Kolattukudy et al., 1995a,b). Consequently, it is possible that the interaction of cutinase with cutin and cutin derived monomers serves as a signal for fungal gene expression associated with pathogenic development.

One of the most abundant classes of cutin monomers is the dihydroxypalmitic acids (DHPAs). Surprisingly, no asymmetric synthesis of a DHPA has been reported (Tulloch, 1980) and the single report assigning the absolute stereochemistry of cutin-derived DHPA is only based on ORD (Espelie and Kolattukudy, 1978; Blée and Schuber, 1993). We now describe the enantioselective synthesis of (*R*)- and (*S*)-10,16-dihydroxyhexadecanoic acid (10,16-DHPA) and the stereochemical assignment of 10,16-DHPA from tomato. The relative ability of the individual enantiomers to elicit transcription of fungal genes in *Colletotrichum trifolii*, the causal agent of alfalfa anthracnose (Dickman, 2000) is also reported.

2. Results and discussion

Our synthetic approach (Scheme 1) was based on the use of a common precursor of both (*R*)- and (*S*)-10,16-DHPA (**1**). The initial portion of the synthesis, illustrated in Scheme 2, began with addition of the lithiated alkyne to 10-undecenal to furnish racemic enynol **2** (Frantz et al., 2000). The derived alkynone **3** underwent reduction with (*R*)- or (*S*)-Alpine borane to furnish enantiomerically enriched propargyl alcohols (*R*)-**2** or (*S*)-**2**, respectively, which were carried individually through the remainder of the synthesis (Singh, 1992). Analysis of derived diastereomeric esters by ^{1}H and ^{19}F NMR (Scheme 3) indicated that (*R*)-**2** and (*S*)-**2** were each formed in at least 88% enantiomeric excess (Dale and Mosher, 1973).

Completion of the synthesis is illustrated in Scheme 4. Ozonolysis under standard conditions (CH_2Cl_2/MeOH, followed by Me_2S reduction) led mainly to the dimethyl acetal. However, ozonolysis in methanol cleanly furnished

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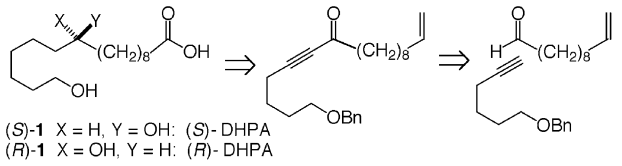
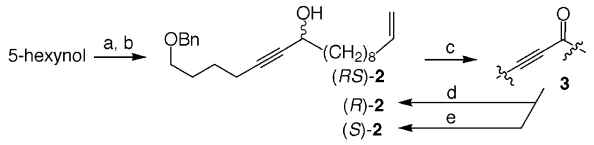
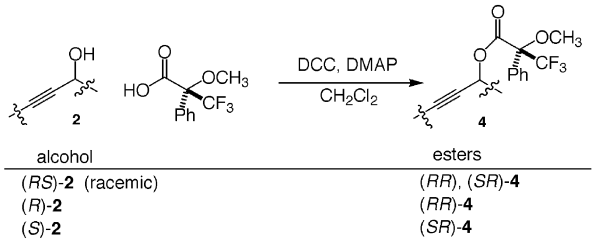


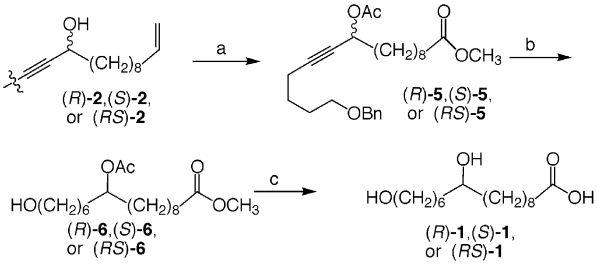
Scheme 1. DHPA structure and retrosynthetic analysis.



Scheme 2. Introduction of the C_{10} stereocenter. (a) NaH, BnBr, DMF (88%); (b) *n*-BuLi, 10-undecenal (86%); (c) PDC (95%); (d) (*R*)-Alpine borane; (e) (*S*)-Alpine borane (90%).



Scheme 3. Determination of stereochemical purity.



Scheme 4. Completion of the synthesis of DHPA. (a) (i) O_3, MeOH; (ii) Ac_2O, pyridine (80%, two steps); (b) Pt/C, H_2 then Pd/C, H_2 (70%); (c) 10% aq. NaOH (96%).

the 1-methoxy-1-hydroperoxyacetal, which was easily fragmented to methyl ester **5** (Schreiber et al., 1982). Sequential hydrogenation over Pt/C and Pd/C saturated the alkyne and deprotected the benzyl ether to furnish **6**. Saponification of both acetate and methyl ester afforded (*R*)-, (*S*)-, and (*RS*)-10,16-DHPA (**1**).

The low magnitude of the optical rotations of (*R*)- and (*S*)-DHPA (**1**), or their methyl esters, led us to seek an alternative method for discriminating the two enantiomers. The near symmetry of 10,16-DHPA (**1**) (the two alkyl chains branching from the C_{10} chiral center are identical for the first six methylene units) appeared to rule out chiral GC or HPLC. Based on a procedure originally applied to racemic dihydroxyoctadecanoates (Sonnet et al., 1994), the (*R*)-, (*S*), and racemic (*RS*) samples of DHPA were individually reacted with an enantiomerically pure chiral isocyanate (Scheme 5). The resulting diastereomeric carbamates **8** could be distinguished by the ^1H NMR signals for the $\underline{Me}_2Si$, *t*-$\underline{Bu}Si$, or the $\underline{Me}O$ groups; details of this analysis are provided in the Experimental section. Although quantitation was limited by partial peak overlap, the assay largely confirmed the enantiomeric purity of the individual synthetic samples of (*R*)- and (*S*)-DHPA. A comparison with the corresponding derivative of tomato-derived 10,16-DHPA (vida infra) demonstrated that the material from tomato possesses mainly the 10(*S*) absolute configuration but also contains a small amount (10–20%) of the 10(*R*)-stereoisomer.

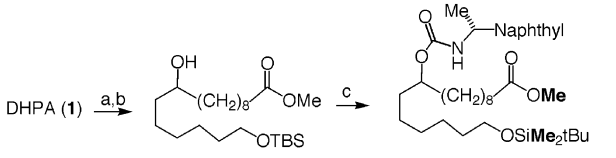
2.1. Isolation and analysis of 10,16-DHPA (*1*) from cutin

Samples of 10,16-DHPA methyl ester were prepared by digestion of tomato cutin using a variant of a reported procedure (Gérard et al., 1992). Silica gel chromatography furnished material which was pure according to GC/MS and which could be saponified to obtain samples of DHPA (**1**). The low magnitude of the optical rotations for individual 10,16-DHPA (**1**) enantiomers rendered specific rotation of only moderate use in determination of stereochemical purity. However, the correlation of the sign of specific rotations for tomato-derived and synthetic samples of DHPA or DHPA methyl ester does substantiate the derivative-based stereochemical assignment described above.

Interestingly, both the synthetic and tomato-derived samples of DHPA (**1**) are stable, while the dihydroxy methyl esters undergo rapid oligomerization. We are currently investigating the basis for this phenomenon, which may be related to the biosynthesis of cutin.

2.2. Influence of cutin monomers on fungal gene expression

Synthetic samples of DHPA (**1**), tomato-derived 10,16-DHPA (**1**) and the known fungal activator aleuritic acid, were compared for their ability to activate a lipid-activated protein kinase (LIPK) and a cutinase both of which are known to be activated by the presence of dihydroxypalmitic acids in the fungal pathogen *C. trifolii,* causal agent of alfalfa anthracnose (Dickman et al., 2003). To date, all major classes of cutins have been shown to be essentially interchangeable with respect to induction of biological activities (Kolattukudy et al., 1995a). RNA was isolated from *C. trifolii* mycelia. Fig. 1 illustrates induction of LIPK after 30 min. (+)-10,16-



Scheme 5. Stereochemical correlation. (a) CH_2N_2, MeOH; (b) *t*-$BuMe_2SiCl$, DMAP; (c) (*R*)-1-naphthylethyl isocyanate.

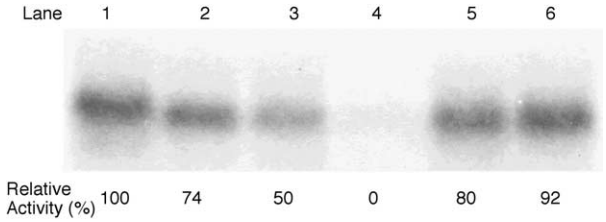


Fig. 1. Relative induction of LIPK activity. Northern analysis of activity of *C. trifolii* LIPK 30 min following treatment. Values are relative to strongest activity. Lane 1: (+)-10,16-DHPA; Lane 2: (−)-10,16-DHPA; Lane 3: (±)-10,16-DHPA; Lane 4: no treatment; Lane 5: cutin-derived 10,16-DHPA; Lane 6: aleuritic acid.

DHPA (**1**) produces slightly greater induction than the (−)-DHPA (**1**), the (+)-DHPA (**1**) derived from hydrolysis of tomato cutin, or aleuritic acid. It is curious that the (+)- and (−)-isomers of 10,16-DHPA (**1**), each consisting largely (>90%) of a single stereoisomer, *each* displayed higher activity relative to the racemic sample.

Fig. 2 displays the corresponding Northern analysis for induction of cutinase 60 min following chemical treatment. In this case, the synthetic and tomato-derived samples of (+)-DHPA (**1**) display similar levels of activity; the (−)-DHPA (**1**) and the racemic DHPA (**1**) are slightly lower. For both the LIPK and cutinase assays, dose-dependent responses were linear for all cases and at all time points (data not shown). Details of the analyses are provided in the Experimental section.

In conclusion, we have reported the first asymmetric syntheses of the enantiomers of 10,16-dihydroxypalmitic acid and have also confirmed the (*S*)(+) absolute stereochemistry of DHPA (**1**) derived from tomato cutin (Espelie and Kolattukudy, 1978). The two DHPA enantiomers are not identical in activation of *C. trifolii* gene expression, suggesting that some aspects of this plant/fungal interaction may involve stereochemical discrimination.

3. Experimental

3.1. General

Standard experimental conditions are described elsewhere (Cho et al., 1999). Unless noted, NMR spectra were recorded in $CDCl_3$ at 500 (^{1}H) and 125 (^{13}C) MHz. ^{1}H NMR peaks are reported as (multiplicity, number of H, coupling constant in Hz).

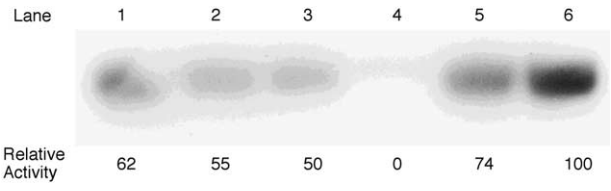


Fig. 2. Relative induction of cutinase activity. Northern analysis of cutinase from *C. trifolii* 60 min following treatment. Values are relative to strongest activity. Lane 1: (+)-10,16-DHPA; lane 2: (−)-10,16-DHPA; lane 3: (±)-10,16-DHPA; lane 4: no treatment; lane 5: natural 10,16-DHPA (see text); lane 6: aleuritic acid.

3.1.1. (*RS*)-1-Benzyloxy-heptadec-16-en-5-yne-7-ol (**2**)

Into a 0 °C solution of 5-hexyn-1-ol (10.1 g, 103 mmol) in 100 ml DMF was added, over 45 min, a 60% suspension of NaH in mineral oil (4.89 g, 204 mmol), followed by benzyl bromide (18.7 g, 109 mmol). The reaction was allowed to warm to room temperature and, after 18 h, was quenched with water (50 ml). The EtOAc extracts were washed with brine and then dried ($MgSO_4$). Following concentration in vacuo, the residue was purified by flash chromatography (5% EtOAc/hexane) to furnish 16.93 g (88%) of the benzyl ether of 5-hexyn-1-ol: R_f = 0.51 (5%EtoAc/hexane); ^{1}H NMR δ 7.46–7.22 (5H), 4.46 (*s*, 2H), 3.45 (*t*, 2H, 6.3), 2.19 (*td*, 2H, 2.6, 7.1), 1.92 (*t*, 1H, 2.7) 1.76–1.57 (*m*, 4H); ^{13}C NMR δ 138.46, 128.20, 127.60, 127.46, 127.42, 127.35, 84.15, 72.70 71.94, 69.46, 68.36, 28.63, 25.10, 18.06; IR 3295, 3088, 3064, 3030, 2940, 2860, 2793, 1496, 1494, 1362, 1112 cm^{-1}; HREI calc. for $C_{13}H_{15}O$ $(M-H)^+$ 187.1126, found 187.1123.

To a −78 °C THF (50 ml) solution of the benzyl ether of 5-hexynol (6.0 g, 33 mmol) was added dropwise a 2.62 M solution of *n*-BuLi in hexanes (13.4 ml, 35 mmol, 1.1 eq). The reaction mixture was stirred for 20 min prior to dropwise addition of 10-undecenal (6.6 ml, 32 mmol, 1.0 eq). The reaction was allowed to warm to RT and stirred for 24 h and then quenched by sequential dropwise addition of methanol (5 ml) and deionised (DI) water (20 ml). The combined hexane and EtOAc extracts were dried over $MgSO_4$. The concentrated residue was purified by flash chromatography (10% EtOAc/hexane) to yield 9.6 g (85%) of the propargyl alcohol: R_f = 0.38 (20% EtOAc/hexane); ^{1}H NMR δ 7.3–7.27 (5H), 5.8 (*ddt*, 1H, 17.1, 10.0, 6.8), 4.95 (*br dd*, 1H, 17.1, 1.8) 4.92 (*br dd*, 1H, 10.0, 1.9), 4.5 (*s*, 2H), 4.33(*t*, 1H, 4.6), 3.49 (*t*, 2H, 6.4), 2.22 (*td*, 2H, 1.9, 7.1), 2.03 (*app q*, 3H, 6.9–7.5), 1.87–1.59 (5H), 1.42–1.28 (12H); ^{13}C NMR (75 MHz) δ 139.88, 139.18, 129.04, 128.28, 128.22, 114.83, 85.61, 82.45, 73.54, 70.44, 63.31, 38.84, 34.49, 30.18, 30.08, 29.97, 29.79, 29.60, 29.53, 26.08, 25.91, 19.18; IR 3396, 3064, 3032, 2928, 2854, 2210, 1672, 1640, 1454, 1360, 1274, 1204, 1104, 1028, 996, 908, 732, 696 cm^{-1}; Anal. calc. for $C_{24}H_{35}O_2$: C, 80.85; H, 10.18; found: C, 80.99; H, 10.32; HRFAB calc. $(M+Li)^+$ 363.2875; found 363.2870.

3.1.2. 1-Benzyloxy-heptadec-16-en-5-yne-7-one (**3**)

A CH_2Cl_2 (5 ml) solution of propargylic alcohol (0.28 g, 0.77 mmol) and pyridinium dichromate (1.5 eq, 0.43 g, 1.1 mmol) was refluxed for 24 h and then filtered through Celite to remove reduced chromium salts. Following removal of solvent at reduced pressure, the crude product was purified by flash chromatography (20%

EtOAc/hexane) to furnish 0.27 g (95%) of the ketone. On a larger scale (11.0 g, 30.8 mmol) the isolated yield was lower (75%). $R_f = 0.70$ (20% EtOAc/hexane); ^{1}H NMR δ 7.33–7.24 (5H), 5.94 (*ddt*, 17.0, 10.3, 6.7), 5.12 (*br d*, 1H, 17), 5.06 (*br d*, 1H, 10), 4.47 (*s*, 2H), 3.47 (*t*, 2H, 6.0), 2.48 (*t*, 2H, 7.4), 2.37 (*t*, 2H, 6.6), 2.02 (*app quarter*, 2H, 7), 1.72–1.61 (6H), 1.36–1.26 (10H); ^{13}C NMR δ 188.34, 139.05, 138.37, 128.56, 128.30, 128.11, 127.65, 127.50, 114.08, 99.59, 93.60, 81.01, 72.86, 69.41, 45.45, 33.70, 29.19, 29.10, 28.96, 28.87, 28.80, 24.56, 24.14, 24.02,18.68; IR 3057, 2934, 2842, 2217, 1669, 1444, 1362, 1106, 983, 922, 737 cm^{-1}; HRFAB calc. $(M)^+$ 354.2559; found 354.2559.

3.1.3. (*R*) or (*S*)-1-Benzyloxy-heptadec-16-en-5-yne-7-ol (**2**) illustrated for (*R*-**2**)

The alkynone (3.64 g, 10.2 mmol) was dissolved in a 0.5 M THF solution of (*R*)-Alpine-borane (41.0 ml, 20.5 mmol). The reaction was stirred for 7 days and then concentrated in vacuo. The residue was redissolved in 100 ml of diethyl ether at 0 °C, and ethanolamine (1.0 ml, 16 mmol) was added dropwise. The resulting suspension was filtered through Celite and concentrated. Gradient flash chromatography (5–20% EtOAc/hexane) furnished 3.4 g (90%) of propargyl alcohol which was identical (^{1}H and ^{13}C) to racemic **2**. $R_f = 0.41$ (20% EtOAc/hexane); $[\alpha]_D = +1.7$ ($c = 3.05$, $CHCl_3$). The (−)-enantiomer was prepared from (*S*)-Alpine borane through the same procedure: $[\alpha]_D = -2.0$ ($c = 1.05$, $CHCl_3$).

3.1.4. Diastereomeric Mosher esters (**4**)

To a solution of propargyl alcohol (*R*)-**2** (12.6 mg, 0.03 mmol) in CH_2Cl_2 (1.0 ml) was added (*R*)-methoxy trifluoromethyl phenylacetic acid (MTPA, 12 mg, 0.05 mmol, 1.5 eq), DMAP (0.25 eq), and dicyclohexylcarbodiimide (11 mg, 0.06 mmol, 1.5 eq), resulting in the formation of a white precipitate. After 2 h, the reaction mixture was diluted with several volumes of ether and then filtered through a plug of cotton. Following removal of solvent, the crude product was filtered through silica (10% EtOAc/hexane) to afford an 82% yield of the MTPA esters, which were determined to be in a 94:6 ratio based upon the ratio of ^{19}F NMR signals at −72.12 and −72.36 ($CDCl_3$). A similar ratio was indicated by the ratio of signals at 3.583 and 3.547 ppm in the ^{1}H NMR spectrum. Analysis of the MTPA esters of (*S*-**4**) prepared similarly, indicated a 6:94 ratio of diastereomers. The corresponding analysis of the racemic alcohol, employed as a control, showed a 51:49 ratio of diastereomers.

3.1.5. Methyl 10-acetoxy-16-benzyloxy-11-hexadecynoate (**5**)

Into a −78 °C solution of alkene **2** (1.05 g, 2.95 mmol) in MeOH (20 ml) tinted slightly pink with a small amount of Sudan Red B was bubbled a gaseous solution of O_3/O_2. Ozonolysis was stopped after the reddish color faded to yellow and little starting material could be observed by TLC. Residual ozone was removed by sparging with N_2 and solvent was removed in vacuo. The residue was redissolved in CH_2Cl_2 (10 ml) and the solution was chilled to −78 °C, whereupon excess acetic anhydride (5 ml) and pyridine (5 ml) were added. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The residue following concentration was subjected to flash chromatography (20% EtOAc/hexane) to furnish the methyl ester (1.01 g, 80% yield) accompanied by 10–15% of the corresponding aldehyde: $R_f = 0.29$ in 20% EtOAc/hexane; ^{1}H NMR δ 7.34−7.25 (5H), 5.33 (*t*, 1H, 6.6), 4.49 (*s*, 2H), 3.66 (*s*, 3H), 3.48 (*t*, 2H, 6.2), 2.29 (*t*, 2H, 7.5), 2.23 (*t*, 2H, 6), 2.05 (*s*, 3H), 1.72−1.58 (8H), 1.58–1.28 (10H); ^{13}C NMR δ 174.70, 170.53, 138.39, 128.77, 128.0, 127.93, 100.09, 86.21, 77.69, 70.18, 64.97, 51.86, 35.50, 34.50, 29.67, 29.55, 29.50, 29.46, 29.25, 25.68, 25.44, 25.33, 22.42, 25.33, 22.42, 21.56, 18.94, 17.98; IR 2924, 2847, 2361, 2225, 1741, 1454, 1362, 1229, 1104, 997, 953, 735, 691 cm^{-1}; Analysis calc. for $C_{26}H_{38}O_5$: C, 72.53, H, 8.90; found: C, 72.36, H, 8.63; HRMS calc. for (M + Li) 437.2879, found 437.2862; (*R*)-**5**: $[\alpha]_D = +39.8$ ($c = 2.7$, $CHCl_3$); (*S*)-**5**: $[\alpha]_D = -37.7$ ($c = 2.4$, $CHCl_3$).

3.1.6. Methyl-10-acetoxy-16-hydroxy hexadecanoate: (**6**)

A mixture of **5** (0.50 g, 1.1 mmol) and 10% Pt/C (30 mol%, 50 mg, 0.33 mmol) in MeOH (10 ml) was hydrogenated in a shaker at 50 psi for 3 days, whereupon 10% Pd/C (30 mol%, 0.33 mmol) was added and the hydrogenation was resumed. After 4 days, the reaction mixture was filtered and solvent removed under reduced pressure. Flash chromatography (40% EtOAc/hexane) furnished 0.35 g (70%) of **6** accompanied by 10–15% of methyl 16-hydroxyhexadecanoate: $R_f = 0.44$ (50% EtOAc/hexane); ^{1}H NMR δ 4.84 (*app quintet*, 1H, 6.6, 5.9, 6.2), 3.6 (*s*, 5H), 2.29 (*t*, 2H, 7.5), 2.05 (*s*, 3H), 1.62–1.50 (8H), 1.33–1.26 (18 H); ^{13}C NMR δ 174.31, 170.94, 74.28, 82.94, 51.43, 34.08, 34.0, 32.65, 29.42, 29.28, 29.22, 29.13, 28.08, 25.59, 25.25, 25.23, 24.91, 23.23, 21.28; IR 3436, 2919, 2858, 2361, 1726, 1557, 1460, 1439, 1367, 1239, 1168, 1050, 1009, 881, 722 cm^{-1}; Anal calc. for $C_{19}H_{36}O_5$: C, 66.24, H, 10.53; Found: C, 66.17, H, 10.88; HRMS calc. for $(M + Li)^+$ 351.2723, found 351.2706. (*R*)−**6**: $[\alpha]_D = -0.28$ ($c = 9.5$, CH_2Cl_2). (*S*)-**6**: $[\alpha]_D = +0.48$ ($c = 7.7$, CH_2Cl_2).

3.1.7. 10, 16-Dihydroxyhexadecanoic acid (10,16-DHPA) (**1**)

A suspension of **6** (191 mg, 0.55 mmol) in MeOH (5 mL) and 10% NaOH (5 ml, excess) was held at 30 °C for 90 min and then acidified to pH 2 with 10% HCl . The suspension was extracted into CH_2Cl_2 and the dried

organic layer was concentrated to give a white solid which was purified by flash chromatography (225:25:1 isopropanol:hexane:acetic acid) to give 10,16 DHPA as a white solid (mp 77–79 °C) which could be further purified by washing with 5% EtOAc/hexane: $R_f = 0.15$ (225:25:1 isopropanol/hexane/acetic acid) or 0.22 (9:1 CH_2Cl_2:MeOH); ^{1}H NMR (3:1 $CDCl_3$:MeOH−d_4) δ 3.37 (*t*, 2H, 6.8), 3.36–3.33 (*m*, 1H) 2.08 (*t*, 2H, 7.5), 1.43–1.11 (26H); ^{13}C NMR δ 176.42, 71.19, 61.88, 48.90, 48.73, 48.56, 48.39, 48.22, 48.05, 47.88, 36.84, 36.73, 32.03, 29.27, 29.11, 29.03, 28.84, 28.74, 25.21, 25.18, 24.57. IR 3460, 3422, 3364, 3328, 3296, 3266, 2922, 2912, 2848, 1700, 1468, 1412, 1284, 1188, 1122, 1058, 1042 cm^{-1}; Analysis calc. for $C_{16}H_{32}O_4$: C, 66.63, H, 11.18; found: C, 62.25, H, 10.74; HRFAB calc. $(M+Li)^+$ 295.2461; found 295.2453. (*R*)-**1**: $[\alpha]_D^{R.T.} = -3.1$ ($c = 0.7$, 1:1 MeOH/$CHCl_3$); (*S*)-**1**: $[\alpha]_D^{R.T.} = +3.0$ ($c = 0.5$, 1:1 MeOH/$CHCl_3$).

3.1.8. Methyl 10,16-dihydroxyhexadecanoate (DHPA methyl ester)

To a solution of 10,16 DHPA (20 mg, 0.066 mmol) in MeOH (10 ml) was added ethereal diazomethane until a yellow tint persisted. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (40% EtOAc/hexane) to furnish the methyl ester (20.7 mg, 99%): mp 47–49 °C; $R_f = 0.3$ (50% EtOAc/hexane); ^{1}H NMR δ 3.67–3.61 (6H), 2.29 (*t*, 2H, 7.5), 1.60–1.24 (26H); ^{13}C NMR δ 174.29, 71.94, 62.99, 51.39, 37.49, 37.38, 34.09, 32.72, 29.58, 29.44, 29.36, 29.15, 29.10, 25.71, 25.58, 24.93; IR 3308, 2924, 2842, 2361, 1731, 1465, 1219, 1127, 1065, 1004, 768, 717 cm^{-1}; Anal calc. for $C_{17}H_{34}O_4$: C, 67.5, H, 11.33; found: C, 68.73, H, 11.37; HRMS calc. $(M+Na)^+$ 325.2355; found 325.2355. (*R*)-**10** $[\alpha]_D = -1.6$ (c = 1.3, $CHCl_3$); (*S*)-**10**: $[\alpha]_D = +1.6$ (c = 1.0, $CHCl_3$).

3.1.9. Methyl-10-hydroxy-16-(dimethyl-1,1-dimethylethyl-silyloxy)hexadecanoate (7)

To a solution of methyl 10,16-DHPA (18 mg, 0.06 mmol) in CH_2Cl_2 (0.5 ml) was added excess Et_3N (0.1 ml), catalytic dimethylaminopyridine (7 mg, 0.06 mmol) and after five min, TBSCl (9 mg, 0.6 mmol) in CH_2Cl_2 (0.3 ml). After 24 h at room temp, the reaction was quenched with saturated ammonium chloride solution and extracted with diethyl ether. The organic layer was washed with brine, dried, and concentrated. The residue was purified by flash chromatography to yield the monosilyl ether in 22% overall yield (5.5 mg) accompanied by the bissilyl ether and recovered starting material (55%): $R_f = 0.33$ (20% EtOAc/hexane); ^{1}H NMR δ 3.66–3.58 (6H), 2.29 (*t*, 2H, 7.52), 1.62–1.25 (25H) 0.89 (*s*, 9H) 0.043 (*s*, 6H); ^{13}C NMR δ 174.32, 71.97, 63.26, 51.43, 37.46, 37.43, 34.09, 32.80, 29.60, 29.49, 29.38, 29.17, 25.97, 25.79, 25.63, 25.60, 24.92, 18.37, 13.57,-5.26; IR 3738, 3390, 2934, 2847, 2361, 1736, 1454, 1250, 1096, 847 cm^{-1}; HRFAB calc. $(C_{23}H_{48}O_4SiLi)^+$ 423.3487; Found 423.3483. (*R*)-**7** and (*S*)-**7** were individually prepared from the (*R*) or (*S*)-enantiomers of methyl 10,16-DHPA by the same procedure.

3.1.10. Formation of diastereomeric carbamates (8)

To a solution of (*RS*)-**7** (2.6 mg, 6.2 μmol) in toluene (0.5 ml), was added (*R*)-(−)-1-(naphthyl)ethyl isocyanate (3.8 mg, 0.02 mmol, 3.3 μl). The reaction was heated to 100 °C for 3 h and then stirred overnight at room temp. Following concentration under high vacuum, the crude product was filtered through flash silica (30% EtOAc/hexane) and directly analyzed by NMR. (*RS*)-**8**: $R_f = 0.81$ (30% EtOAC/hex); ^{1}H NMR δ 7.86–7.43 (*m*, 7H), 3.667, 3.66 (*s*, 3H), 3.625–3.56 (*m*, 1H), 2.3–2.16 (7H), 1.67–1.25 (33H), 0.895/0.885 (*s*, 9H) 0.048/0.035 (*s*, 6H); ^{13}C NMR δ 174.32, 155.84, 128.79, 126.34, 125.70, 125.23, 122.06, 114.28, 74.95, 63.25, 51.43, 34.48, 34.10, 32.77, 29.69, 29.49, 29.39, 29.16, 29.10, 25.98, 25.71, 25.25, 24.93, −5.26; IR 2924, 2847, 2356, 2330, 1740, 1710, 1644, 1629, 1562, 1506, 1541, 1378, 1244, 1091,1050, 835, 768 cm^{-1}; HRFAB calc. for $(C_{36}H_{59}NO_5SiLi)^+$ 620.4323; found 620.4328.

The corresponding diastereomers derived from (*R*)-**7** and (*S*)-**7** could be distinguished by small differences in the singlets at δ 3.66 (OCH_3), 0.89 (*t-Bu*Si), or 0.18 (MeSi) ppm in the ^{1}H NMR. A mixture of the naphthyl carbamates derived from synthetic (*R*)-DHPA and tomato-derived DHPA displayed two sets of signals; the corresponding mixture involving (*S*)-DHPA gave one major set of signals.

3.2. Isolation of cutin monomer

Cutin polymers were isolated using a streamlined modification of a published procedure (Gérard et al., 1992). Small sections of peel from mature tomatoes (1.42 kg) were washed in DI water and then air dried. The peels were boiled in 1 l of oxalate buffer (4 g oxalic acid and 16 g ammonium oxalate) for 90 min and washed with DI water. The dried peels were subjected to overnight Soxhlet extraction with $CHCl_3$ and the residue was dried under vacuum. Washing with water and vacuum drying resulted in 2 g of a yellow-brown powder.

The cutin polymer (0.5 g) was refluxed for 2 days in a solution of 14% (v/v) BF_3 /MeOH (66 ml), whereupon the cooled reaction was carefully quenched with saturated bicarbonate (150 ml). Following extraction with CH_2Cl_2 (3×50 ml) and drying with Na_2SO_4, concentration in vacuo furnished 0.3 g of a viscous orange liquid. The crude product was dissolved in 50% EtOAc/hexane and subjected to silica gel flash chromatography (50% EtOAc/hexane) to furnish the methyl ester of 10,16-DHPA as an off-white/yellow powder which displayed NMR and IR spectra identical to synthetic material: mp 48–49 °C; $[\alpha]_D = +0.6$ ($c = 1$, $CHCl_3$).

Solutions of methyl DHPA appeared to undergo rapid polymerization or oligomerization. Optical rotations were acquired on freshly purified material using alumina-filtered $CHCl_3$.

3.2.1. DHPA (*1*) from cutin

DHPA methyl ester, derived as described above, was saponified to furnish DHPA (**1**), which displayed NMR and IR spectra identical to synthetic material. The melting point differed slightly from synthetic DHPA, with deformation occurring at 64–65 °C followed by complete melting at 74–75 °C; $[\alpha]_D + 3.1$ ($c = 0.5$, 1:1 MeOH/$CHCl_3$).

3.2.2. RNA analysis

Fungi were grown in liquid shake culture in 0.2% glucose (limiting) for 3 days, after which DHPA (sonicated in water) was added. The RNA was isolated at selected time points following this treatment (Podilla et al., 1988).

For Northern hybridization, aliquots (10 μg) of RNA were mixed with 0.48 μg ethidium bromide, 1 μl formamide and 1 μl 10×MOPS buffered [0.2 M 3-(*N*-morpholino) propanesulfonic acid, 80 mM sodium acetate pH 7, 10 mM EDTA, 3 M formaldehyde] in 10 μl volumes. Samples were heated at 75 °C for 5 min prior to addition of 1.1 μl 10×loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 25% Ficoll, 10 mM EDTA) and loading on 1% agarose gels in 1×MOPS buffer. Gels were run at 3 V/cm for 3.5 h in 1×MOPS buffer. Gels were soaked twice in 2×SSPE for 15 minutes each. RNA was blotted onto MagnaGraph nylon filters (MSI) with 20×SSPE for 16 h and subsequently fixed by *uv* irradiation. Blots were hybridized in 0.25 M dibasic sodium phosphate pH 7.4, 7% SDS, 2% blocking reagent (Boehringer Mannheim) and 1 mM EDTA. A 1.4-kb *Bam*HI-*Eco*RI fragment encoding 17S ribosomal DNA from *Neurospora crassa* was labeled with digoxigenin-dUTP by random priming and served as an internal control to ensure equivalent loadings. RNA signals were normalized relative to 17S ribosomal *N. crassa* DNA signals by computer imaging and analysis using Collage software by Fotodyne. Hybridizations included 25 ng probe in 10 ml solution at 65 °C for 20 h. Filters were washed at high stringency including two washes with 0.2×SSPE, 0.1% SDS at 65 °C for 20 min each. Procedures for digoxigenin detection with the chemiluminescent substrate Lumi-Phos 530 were according to the manufacturer's (Boehringer Mannheim) instructions. Analyses were done in triplicate.

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