Synthesis of Mayolene-16 and Mayolene-18: Larval Defensive Lipids from the European Cabbage Butterfly

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A tandem Wittig approach has been employed for the synthesis of both (11S,9Z,12Z,15Z)- and (11R,9Z,12Z,15Z)-hydroxyoctadeca-9,12,15-trienoic acid (11-hydroxylinolenic acid, 11-HLA) from (R)-glyceraldehyde acetonide. From (11R)-HLA we have prepared the corresponding palmitic acid and stearic acid esters, mayolene-16 (1) and mayolene-18 (2), insect defensive compounds recently identified from Pieris rapae larvae. In addition, we describe the synthesis of three macrocyclic oligomers (24–26) derived from (11R)-HLA.

Introduction

As part of our ongoing investigation of the chemical defenses of insect larvae and pupae, we have recently characterized a new family of biologically active lipids, the mayolenes, from the glandular hair secretion of larvae of the European Cabbage butterfly, Pieris rapae.1 The mayolenes consist of (11R)-HLA2 (11-HLA = 11-hydroxylinolenic acid) esterified with a series of homologous saturated fatty acids. The major components of this larval defensive secretion, mayolene-16 (1) and mayolene-18 (2), are (11R)-HLA esters of palmitic acid and stearic acid, respectively.

Following the structural characterization of the mayolenes, we were interested in synthesizing the individual enantiomers of 11-HLA, which we needed to determine the absolute configuration of these natural products, as well as to provide samples of the mayolenes for further study of their biological activity.1 At an early stage in the course of characterizing the mayolenes, we considered candidate structures 24–26, structural analogues of the polyazamacrolides recently identified from ladybird beetle pupae.3 To establish the presence or absence of these macrocyclic lactones in the insect secretion and to study their biological activity, we wished to prepare compounds 24–26 as well. We now report the synthesis of both enantiomers of 11-HLA, mayolene-16 (1) and mayolene-18 (2), and of the oligomeric macrolides 24–26.

Results and Discussion

Because the bis-allylic hydroxyl group in 11-HLA is particularly prone to 1,4-elimination, we sought syntheses of (11R)- and (11S)-HLA compatible with the acid- and heat-sensitive nature of the intermediates and product. Our route utilizes two consecutive Wittig reactions to construct the C5-C10 and C12-C13 cis double bonds, and allows both enantiomers of 11-HLA to be prepared from (R)-glyceraldehyde acetonide (9) simply by varying the order in which the two Wittig reactions are carried out (Scheme 1).

To prepare (11R)-HLA, the ylide derived from (3Z)-3-hexyltriphenylphosphonium bromide (6)4 was treated with 9,5 providing diene 10 with excellent diastereoselectivity (Z:E > 99:1, GC).6 Deprotection of the acetonide using methanolic hydrochloric acid afforded diol 11 (Scheme 2).

Conversion of 11 to the corresponding di-tert-butylidimethylsilyl ether, followed by anticipated regioselective deprotection of the primary silyl ether using HF–pyr7 surprisingly gave only the undesired product, arising from selective removal of the secondary, allylic silyl ether. To circumvent this problem, diol 11 was regioselectively protected as the primary tert-butylidimethylsilyl ether 12 using TBSCI and triethylamine in the presence of DBU.8

The secondary hydroxyl group in silyl ether 12 was protected as the tert-butylidiphenylsilyl ether to provide 13, followed by selective deprotection of the primary silyl ether in the presence of PPTS, affording alcohol 14. Interestingly, the tert-butylidiphenylsilyl group in 14 was found to migrate slowly to the primary hydroxyl group. Oxidation of freshly prepared 14 using tetrapropyliummonium permanganate/4-methyImorpholine N-oxide (TPAP/NMO)\(^\text{8}\) afforded aldehyde 7, which was subsequently treated with the silyl-protected (8-carboxymethylloctyltriphenylphosphonium bromide(5)\(^\text{9}\) to yield trienoate 15. Treatment of 15 with TBAF afforded alcohol 16, which upon hydrolysis with aqueous lithium hydroxide followed by careful neutralization, provided (11R)-HLA (3).

To prepare (11S)-HLA, 9 was treated with the ylide derived from 5 to afford 17 with excellent diastereoselectivity (Z:E > 98:2, GC). Deprotection of 17 with methanolic hydrochloric acid afforded diol 18 (Scheme 3). Protection of the primary hydroxyl group as the tert-butylidimethylsilyl ether gave 19. The free secondary hydroxyl group of 19 was protected as the tert-butylidiphenylsilyl ether to afford 20, which was regioselectively deprotected at the primary hydroxyl group to yield alcohol 21. Oxidation of 21 with PCC provided aldehyde 8. A Wittig reaction between 8 and the ylide prepared from 6 afforded triene 22, which was subsequently deprotected using TBAF, to yield alcohol 23. Hydrolysis of 23 provided (11S)-HLA (4). With synthetic samples of optically pure (11R)- and (11S)-HLA in hand, we showed the absolute configuration of the mayolones to be (11R).\(^\text{1}\)


Reagents and conditions: (a) LiHMDS, −78 °C, THF, then 9; (b) HCl, MeOH; (c) TBSi, Et3N, DBU, CH2Cl2; (d) TBDPSi, DMAP, CH2Cl2; (e) PPTS, EtOH; (f) PCC, NaOAc, CH2Cl2; (g) 6, LiHMDS, −78 °C, THF, then 8; (h) TBAF, THF; (i) LiOH, MeOH.

Reagents and conditions: (a) EDCI, DMAP, CH2Cl2, then 3.

As products, we found that 2,4,6-trichlorobenzoyl chloride/Et3N/DMAP15 yielded predominantly the desired macrocycles 24−26 (Scheme 5), along with small amounts of tetramer and pentamer macrocycles. Similar experience during the total synthesis of mueggelone,16 an allylic 10-membered macrocycle independently isolated by two groups from cyanobacterial blooms, has been reported. Only by employment of 2,4,6-trichlorobenzoyl chloride/Et3N/DMAP was successful macrocyclization of the appropriate precursor to mueggelone accomplished.17

Chromatography of the macrocyclization products formed from (11R)-HLA provided samples of pure 24−26, along with the tetramer, and a sample of a pentamer admixed with small amounts of the tetramer and higher oligomers. The 1H NMR spectra of macrocycles 24−26 are readily distinguishable from each other and from the natural secretion. While the 1H NMR spectra of the tetramer and pentamer are virtually indistinguishable, and resemble some relevant portions of the 1H NMR spectra of O-acyl derivatives of the acyclic monomer (11R)-HLA, the C-11 proton chemical shift value in these macrocycles is clearly upfield of the corresponding C-11 proton in O-acyl derivatives of (11R)-HLA, indicating that none of these macrocycles are present in the glandular hair secretion.

In summary, we have described a concise route to both enantiomers of 11-HLA from (R)-glyceraldehyde ac-
etoxide (9) and the Wittig reagents prepared from phosphonium ylides 5 and 6. From (11R)-HLA, we have prepared mayolene-16 (1) and mayolene-18 (2), both of which exhibit strong insect-deterrent activity in bioassays. To facilitate chemical characterization of the Pieris rapae secretion, we also synthesized several macrolides derived from (11R)-HLA. These macrolides turned out not to be part of the larval armamentarium.

Experimental Section

General Information. All commercially available reagents were used without further purification. Solvents were either purchased or distilled using common practices where appropriate. All reactions were carried out under dry nitrogen. TLC was performed using 2.5 x 7.5 cm plates precoated with silica gel (200 μm), and spots were visualized by anisaldehyde. Silica gel (60 Å) was used for flash column chromatography. An aliquot of aqueous ammonium hydroxide was added to solutions of mayolene-16 (1) and mayolene-18 (2) prior to positive ion electrospray mass spectrometry.

(4S,1’Z,4’Z)-2,2-Dimethyl-4-hepta-1,4-dien-1-yl-1,3-dioxolane (10). A 1.0 M solution of lithium bis(trimethylsilyl)amide (4.22 mL, 4.22 mmol) in THF was added dropwise over 30 min to a stirred solution of 6 (1.96 g, 4.61 mmol) in THF (8 mL) at 0 °C. After being stirred for 30 min at 0 °C, the deep orange solution was cooled to ~78 °C, and to the solution was added dropwise a solution of 9 (500 mg, 3.84 mmol) in THF (3 mL). The solution was stirred for 3 h at 0 °C, at which time the reaction was quenched by slow addition of a saturated aqueous NH₄Cl solution (30 mL). After brief stirring, the yellow liquid was extracted with CH₂Cl₂ (45 mL). Combined organic extracts were dried (MgSO₄), filtered, and concentrated under vacuum. Flash chromatography (30:1 hexane/ethyl acetate) of the residual orange oil provided 10 (520 mg, 69%) as a clear oil.

(2S,3’Z,6’Z)-3,6-Nonadiene-1,2-diol (11). Concentrated aqueous hydrochloric acid (3.3 mL) was added dropwise to a stirred solution of 10 (2.03 g, 10.3 mmol) in methanol (30 mL) at 0 °C. After the solution was stirred for 4 h at 0 °C, the pH was adjusted to 7 by slow addition of an aqueous NH₄OH solution (25%, v/v). After methanol was removed under vacuum, the solution was diluted with water (15 mL) and extracted with ether (110 mL). Combined organic extracts were dried (MgSO₄), filtered, and concentrated under vacuum. Flash chromatography (1:1 hexane/ethyl acetate) of the residual yellow oil provided 11 (1.45 g, 90%) as a light yellow oil.

(2S,3’Z,6’Z)-1-[(tert-Butyldimethylsilyl)oxy]-3,6-nonadien-2-ol (12). Triethylamine (0.045 mL, 0.33 mmol), DBU (0.010 mL, 0.065 mmol), and tert-butyldimethylsilyl chloride (50 mg, 0.33 mmol) were added to a stirred solution of 11 (51 mg, 0.33 mmol) in CH₂Cl₂ (0.5 mL). After being stirred for 6 h at rt, the solution was washed with 10 mL of ice cold aqueous HCl (5%, v/v) and extracted with CH₂Cl₂ (40 mL). The combined organic layers were washed with a saturated aqueous NaHCO₃ solution (15 mL), dried (MgSO₄), filtered, and concentrated under vacuum. Flash chromatography (15:1 hexane/ethyl acetate) of the residual oil provided 12 (75 mg, 85%) as a clear oil.

(2S,3’Z,6’Z)-1-[(tert-Butyldimethylsilyl)oxy]-2,3-tert-butyldiphenylsilyl)oxy)-3,6-nonadiene (13). DMAP (440 mg, 3.60 mmol) and tert-butyldiphenylsilyl chloride (0.93 mL, 3.60 mmol) were added to a stirred solution of 12 (487 mg, 1.80 mmol) in CH₂Cl₂ (10 mL). After being stirred for 18 h at rt, the solution was diluted with brine (25 mL) and extracted with CH₂Cl₂ (75 mL). Organic extracts were combined, dried (MgSO₄), filtered, and concentrated under vacuum. Flash chromatography (60:1 hexane/ethyl acetate) of the residual oil provided 13 (88 mg, 99%) as a clear oil.

Synthesis of Mayolene-16 and Mayolene-18

Mayolene-16 (1) [11S,Z12,Z15,Z12]-1-Hexadecanoyloxyoctadeca-9,12,15-trienoic Acid]. A solution of palmitic acid (14.4 mg, 0.056 mmol) in CH₂Cl₂ (0.25 mL) was treated with DMAP (6.9 mg, 0.056 mmol) and EDCI (10.7 mg, 0.056). The resulting cloudy solution was stirred for 10 h at rt followed by the addition of a solution of 3 (15.0 mg, 0.051 mmol) in CH₂Cl₂ (0.10 mL). After the solution was stirred for 12 h, the solvent was removed under vacuum. The residue was dissolved in NaOAc buffer (10 mL, 0.4 M, pH 5.75) and extracted with

ether. Combined organic extracts were dried (MgSO4), filtered, and concentrated under vacuum. Flash chromatography (5.1 hexane/ethyl acetate) of the residual oil provided 1 (16.0 mg, 59%) as a white solid: [α]D −1.5 (c 0.84, CHCl3); Rf = 0.47 (3:1 hexane/ethyl acetate); 1H NMR (500 MHz, benzene-d6) δ 6.74–6.80 (m, 1 H), 5.58–5.64 (m, 2 H), 5.48–5.54 (m, 2 H), 5.41–5.46 (m, 2 H), 5.32–5.35 (m, 1 H), 5.22–5.26 (m, 1 H), 2.16–2.25 (m, 3 H), 1.84–2.12 (m, 1 H), 1.47–1.64 (m, 4 H), 1.12–1.37 (m, 32 H), 0.93 (m, 3 H), 0.92 (t, J = 7.2 Hz, 3 H); 13C NMR (100 MHz, benzene-d6) δ 179.2, 172.3, 133.9, 132.9, 128.0, 127.6, 66.3, 34.5, 34.0, 32.3, 30.16, 30.15, 30.13, 30.12, 30.11, 30.10, 30.08, 30.00, 29.81, 29.80, 29.7, 29.5, 29.2, 28.3, 26.7, 25.3, 24.9, 23.1, 20.9, 14.4, 14.3; HRMS (ESI) m/z 550.4812 (550.4835 calculated for CsH64NO16 (M + NH4)).

Mayenole-18 (2) [(1S,9Z12Z15Z)-11-Octadecanoyloxy-octadeca-9,12,15-trienoic Acid]. A solution of stearic acid (21.2 mg, 0.074 mmol) in CH2Cl2 (0.30 mL) was treated with DMAP (0.1 g, 0.074 mmol) and EDCI (14.3 mg, 0.074). The resulting cloudy solution was stirred for 12 h at rt followed by the addition of a solution of 3 (20.0 mg, 0.068 mmol) in CH2Cl2 (0.10 mL). After the solution was stirred for 15 h, the solvent was removed under vacuum. The residue was dissolved in NaOAc buffer (10 mL, 0.4 M, pH 5.75) and extracted with ether. Combined organic extracts were dried (MgSO4), filtered, and concentrated under vacuum. Flash chromatography (5.1 hexane/ethyl acetate) of the residual oil provided 2 (22.4 mg, 59%) as a white solid: [α]D −1.4 (c 0.90, CHCl3); Rf = 0.51 (3:1 hexane/ethyl acetate); 1H NMR (500 MHz, benzene-d6) δ 6.74–6.80 (m, 1 H), 5.58–5.64 (m, 2 H), 5.48–5.54 (m, 2 H), 5.41–5.46 (m, 2 H), 5.32–5.35 (m, 1 H), 5.22–2.16 (m, 3 H), 2.04–2.12 (m, 4 H), 1.57–1.64 (m, 4 H), 1.12–1.37 (m, 36 H), 0.93 (m, 3 H), 0.92 (t, J = 7.2 Hz, 3 H); 13C NMR (100 MHz, benzene-d6) δ 179.2, 172.3, 133.9, 132.9, 128.0, 127.6, 66.3, 34.5, 34.0, 32.3, 30.18, 30.16, 30.14, 30.11, 30.10, 30.09, 30.01, 29.82, 29.80, 29.59, 29.29, 28.2, 26.6, 25.3, 24.9, 23.1, 20.9, 14.4, 14.3; HRMS (ESI) m/z 578.5138 (578.5148 calculated for CsH68NO17 (M + NH4)).

Macrolides 24–26. A solution of 3 (28.5 mg, 0.097 mmol) in benzene (2.0 mL) was treated with PyrNET (86.0 mL, 0.48 mmol) and 2,4,6-trichlorobenzoyl chloride (75.0 mL, 0.48). The resulting solution was stirred for 3 h at rt and then rapidly cannulated into a solution of DMAP (177 mg, 1.45 mmol) in benzene (25 mL). After being stirred for 14 h, the solution was diluted with ether (25 mL), filtered through Celite, and concentrated under vacuum. Flash chromatography (CHCl3) of the residual oil on C18-modified silica gel provided 24 (11.8 mg, 44%), 25 (9.5 mg), and 26 (2.2 mg) as clear oils.

Data for [10Z]-12-[(1Z,4Z)-Hepta-1,4-dienyl]-1-oxyacyclodec-10-en-2-one (24): 1H NMR (500 MHz, benzene-d6) δ 6.60 (m, J12,11 = J12,1′ = 8.2, 1 H, 12-H), 5.63 (m, J10,11 = 10.8, J10,11 = 8.2, 1 H, 11-H), 5.39–5.49 (m, 2 H, 4′-H and 5′-H), 5.49–5.57 (m, 2 H, 1′-H and 2′-H), 5.35 (m, J10,11 = 10.8, J10,11 = 12, J10,11 = 4.5, 1 H, 10-H), 3.10–3.20 (m, 2 H, 3′-H), 2.39 (m, J12,11 = 13, J12,11 = 3, J12,11 = 11.7, J12,11 = 3, 1 H, 9-Hα), 2.15 (dd, J12,11 = 14, J12,11 = 2.5, J12,11 = 7.5, 1 H, 3-Hqα), 2.05 (m, J12,11 = 7.5, 1 H, 2′-H), 2.02 (dd, J12,11 = 7.5, 1 H, 3-Hqα).

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Supporting Information Available. Analytical data for compounds 7 and 10–16, experimental procedures and analytical data for compounds 4, 8, and 17–23, and spectroscopic data of the naturally occurring mixture of mayenoles. This material is available free of charge via the Internet at http://pubs.acs.org.