Macrolides from the scent glands of the tropical butterflies *Heliconius cydno* and *Heliconius pachinus*†

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The four major components present in scent gland extracts of the male Costa Rica longwing butterflies *Heliconius cydno* and *Heliconius pachinus* were identified as 12- and 14-membered macrolides containing a C_{18}-carbon skeleton. By use of micro-reactions and spectrometric examinations, structural proposals were made and subsequently proven by synthesis, using ring-closing-metathesis as the key steps. These macrolides, (9*Z*,11*E*,13*S*)-octadeca-9,11-dien-13-olide (**5**, *S*-coriolide), (9*Z*,11*E*,13*S*,15*Z*)-octadeca-9,11,15-trien-13-olide (**6**), (9*Z*,13*S*)-octadec-9-en-13-olide (**13**), and (9*Z*,11*S*)-octadec-9-en-11-olide (**25**), are biosynthetically obviously derived from oleic, linoleic, and linolenic acids. Their absolute configurations were determined by gas chromatographic investigations on chiral phases, showing all to possess (*S*)-configuration.

Introduction

The Costa Rica longwing butterflies *Heliconius cydno* and *H. pachinus* (Lepidoptera, Nymphalidae) are members of the Central- and South-American Heliconiinae subfamily. These butterflies are known for their complex mimicry rings and interesting behaviour.[1]

Gilbert postulated that males of a related species, *H. erato*, transfer a pheromone, a so-called antiaphrodisiac, during copulation to the female which deters other males from this female.[2] Chemical investigations of another heliconiid, *Agraulis vanillae*, showed that both males and females contain the same compounds in their scent bouquet. Ross *et al.*[3] identified 6-methyl-5-hepten-2-one and fatty acid esters of the corresponding alcohol as well as 1,15- and 1,16-hexadecanediyl diacetates in their secretion. They postulated that the secretion functions as a deterrent against predators, but no experimental proof was given. Meinwald and coworkers isolated a fatty acid derived macrolide, (*R*)-coriolide (*R*-**5**), from both sexes of *H. pachinus*, a species closely related to *H. cydno*,[4] and suggested a pheromonal role of this compound.[5]

Recently these butterflies have received considerable attention in terms of the molecular genetics of wing colour pattern, genetics of mate preference and speciation,[6] so additional details of the chemistry of pheromonal secretions adds to an important model system in evolutionary biology. Both *H. pachinus* and *H. cydno* hybridise with an exchange of genes with *H. melpomene*,[7] a species distinct in both colour pattern and pheromonal chemistry.[8] Thus this system presents mysteries only understood when chemistry, ecology, and behaviour are unravelled in concert.

In the present work the synthesis, identification, and enantiomer determination of coriolide and three additional major macrolides occurring in the scent glands of male and female *H. cydno* and *H. pachinus* are reported, and their function is discussed in relation to the behaviour of the butterflies.

Results and discussion

Abdominal scent glands of male *Heliconius cydno* and *H. pachinus* raised in a greenhouse were cut and extracted with pentane. The extracts were analysed by GC-MS revealing the presence of four major compounds **A–D** (Fig. 1). The mass spectrum of compound **C** (Fig. 2) was almost identical to that reported for (9*Z*,11*E*)-octadeca-9,11-dien-13-olide (coriolide, **5**).[5,9]

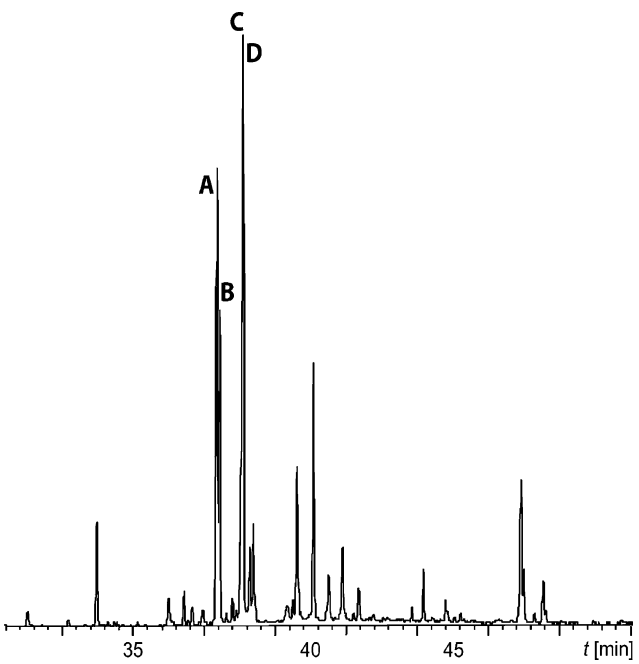

Fig. 1 Total ion chromatogram of an extract of male abdominal scent glands of *Heliconius cydno*. The chromatogram of *H. pachinus* is very similar.

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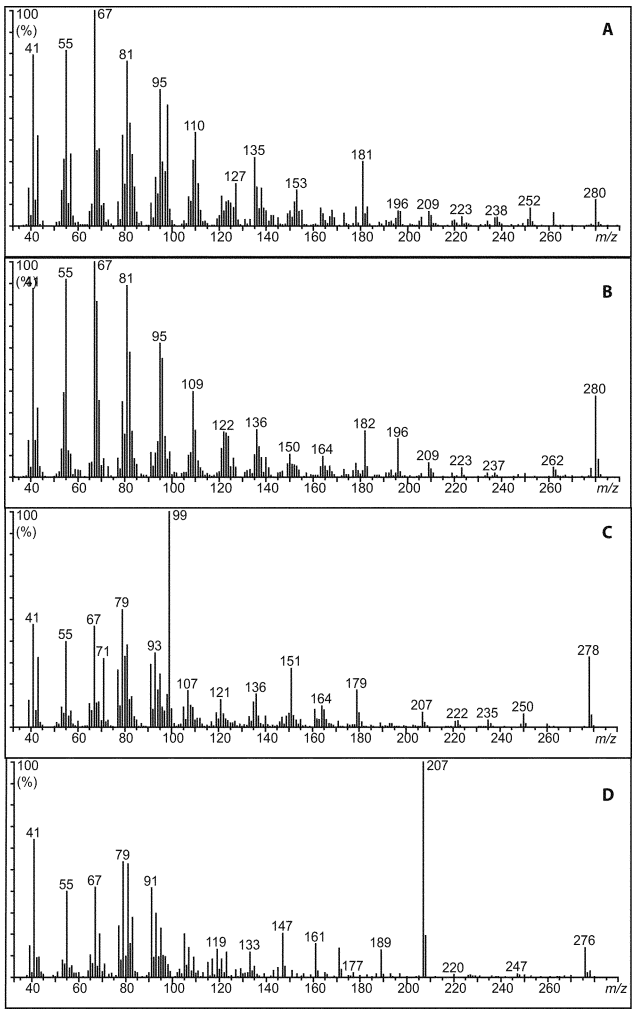


Fig. 2 Mass spectra of compounds **A** ((*Z*)-octadec-9-en-11-olide, *Z*-**25**), **B** ((*Z*)-octadec-9-en-13-olide, **13**), **C** ((9*Z*,11*E*)-octadeca-9,11-dien-13-olide, coriolide, **5**), and **D** ((9*Z*,11*E*,15*Z*)-octadeca-9,11,15-trien-13-olide, **6**).

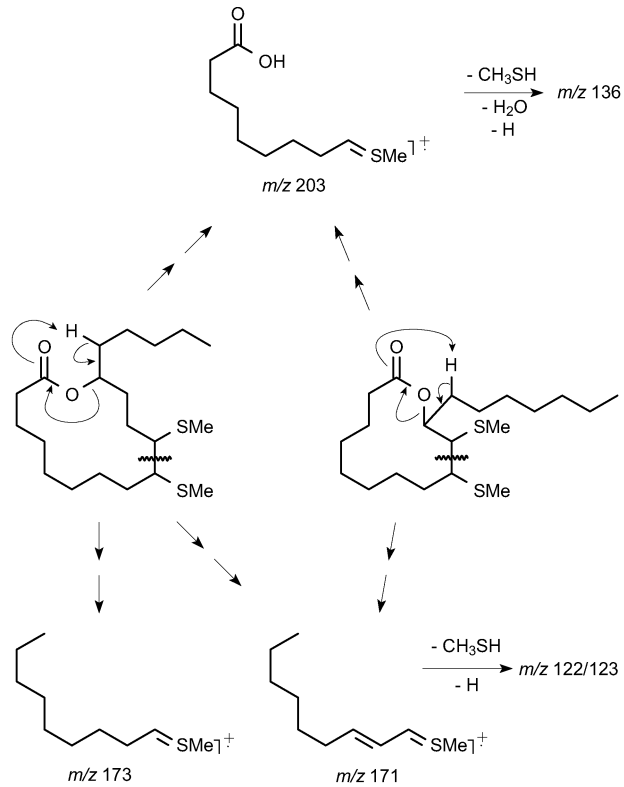
The other three compounds exhibited similar mass spectra, but with different molecular ions. Compounds **A** and **B** had a molecular mass 2 amu higher than that of **C**, suggesting these to be two octadecenolides, while compound **D** had a molecular mass 2 amu less than that of **C** and was therefore believed to be an octadecatrienolide. The mass spectrum of **5** showed a characteristic ion at *m*/*z* 207 (M − 71), which can be attributed to the loss of the pentyl side chain. This ion occurs also prominently in the spectrum of compound **D**, in which case it corresponds to the loss of a pentenyl side chain (M − 69). Compound **B** showed a small ion at *m*/*z* 209, which could be interpreted as a loss of a pentyl side chain. In contrast, the mass spectrum of compound **A** contained an intense ion at *m*/*z* 181 (M − 99) pointing to a heptyl side chain. Hydrogenation of an extract with Pd/C furnished two saturated macrolides **E** and **F** (for mass spectra see ESI†). The main one **E** was identified as 13-octadecanolide by its characteristic ion at *m*/*z* 211 (M − 71), while the minor one showed a peak at *m*/*z* 183 (M − 99), consistent with 11-octadecanolide. Biosynthetically, coriolide is certainly derived from linoleic acid by oxidation at C-13 with concomitant double bond migration, followed by ring closure. A similar mechanism starting with linolenic acid would lead to (9*Z*,11*E*,15*Z*)-octadeca-9,11,15-trien-13-olide, the putative structure of compound **D**. Compounds **A** and **B** are most likely derived from oleic acid, but a different precursor like vaccenic acid seemed also to be plausible.

We therefore tried to elucidate the position of the double bond in both octadecenolides by addition of dimethyldisulfide (DMDS) to the extract, followed by GC-MS analysis.[10,11] Surprisingly, the adducts showed largely different mass spectra (Fig. 3). The adduct of **A** exhibited an intensive cluster of peaks around *m*/*z* 171. This can be explained by elimination of the ester group from the chain and additional loss or transfer of hydrogen. When the double bond is located at C-9, cleavage between the two thiomethyl groups leads to such ions carrying the alkyl end of the molecule. The other cleavage product carrying the carboxyl end is also present at *m*/*z* 203. Ions at *m*/*z* 171 and 203 can be found in the spectrum of the second adduct, too, but only of relatively low abundance. Instead, ions at *m*/*z* 122 and 136 dominate. They can be explained by additional losses of CH_3SH, H_2O, and H from *m*/*z* 171 or 203, as shown in Fig. 3. These results pointed towards the location of the double bond at C-9 in both compounds, but other positions could not be completely ruled out by the derivatisation results.

The macrolides **A**–**D** were then synthesised to prove our structural assignments, to elucidate their absolute configuration, and provide material for biotests. (*S*)-Coriolide (**5**) has been synthesised before using the enzymatic oxidation of linoleic acid (**2**) with soy bean lipoxygenase, reduction and macrolactonisation,[12,13] while a similar reaction with linolenic acid (**1**) leading to octadeca-9,11,15-trien-13-olide (**6**) was described in a patent.[14] We followed this route using both **2** and **1** as starting materials. The oxidation with soy bean lipoxygenase at C-13 proceeds with concomitant double bond migration and is known to furnish the *S*-configured hydroperoxide.[15,16] Reductive work-up with $NaBH_4$[15] delivers the corresponding acids (9*Z*,11*E*,13*S*)-13-hydroxyoctadeca-9,11-dienoic acid (coriolic acid, **3**) and (9*Z*,11*E*,13*S*,15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (**4**). Cyclisation worked best in our hands using Corey/Nicolaou conditions[13] to furnish *S*-**5** and *S*-**6** (Fig. 4). Both macrolides were generated in high ee (>95%), but only moderate yield. Nevertheless, the target molecules are obtained in two steps only, making this route very attractive for this class of compounds. The two macrolides were identical to the natural compounds, thus confirming our structural assignments for **C** and **D**. Non-racemic mixtures of their enantiomers were then needed for the determination of the absolute configuration of the natural compounds. They were synthesised by inversion of *S*-**3** and *S*-**4** by the Mitsunobu procedure, followed by macrolactonisation.

Gas chromatographic analysis of the synthetic macrolides **5** and **6** and a crude scent gland extracts of *Heliconius cydno* and *Heliconius pachinus* on a chiral Hydrodex (2,3-di-*O*-methyl-6-TBDMS-β-cyclodextrin) phase showed that both natural macrolides are enantiomerically pure and possess *S*-configuration (Fig. 5). Therefore, compound **C** is (9*Z*,11*E*,13*S*)-octadeca-9,11-dien-13-olide (*S*-**5**, coriolide), while the structure of compound **D** is (9*Z*,11*E*,13*S*,15*Z*)-octadeca-9,11,15-trien-13-olide (*S*-**6**).

Our attention then turned towards the synthesis of the two octadecenolides **A** and **B**. Attempts to apply the soy bean lipoxygenase reaction to oleic acid for synthesis of the two compounds failed. Therefore we switched to a synthetic route using



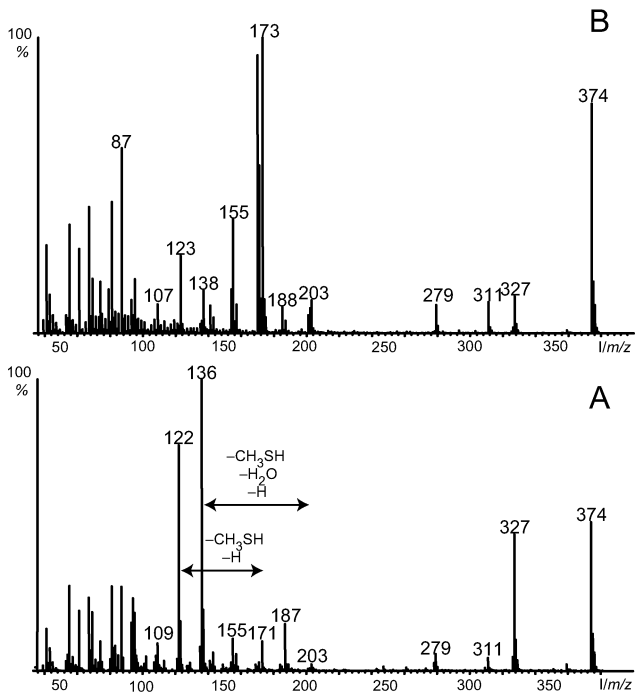


Fig. 3 Mass spectra and fragmentation pattern of DMDS-adducts derived from **A** and from **B**.

ring-closing-metathesis (RCM) as the key step. The Grignard reaction of hexanal (**7**) and 1-bromo-3-butene furnished dec-1-en-5-ol **9**, which was acylated with 9-decenoic acid (**11**), generated from 9-decen-1-ol (**10**). The resulting ester *rac*-**12** was then submitted to RCM using the Grubbs' catalyst of the first generation (Fig. 6). *rac*-Octadec-9-en-13-olide (**13**) was obtained in good

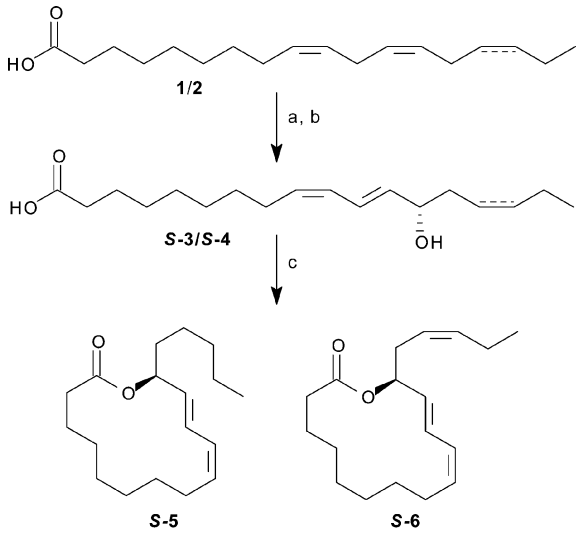


Fig. 4 Synthesis of coriolide ((*S*)-**5**) and (*S*)-**6**: a) soybean lipoxygenase, O_2, b) $NaBH_4$, c) PPh_3, $(C_5H_5NS)_2$.

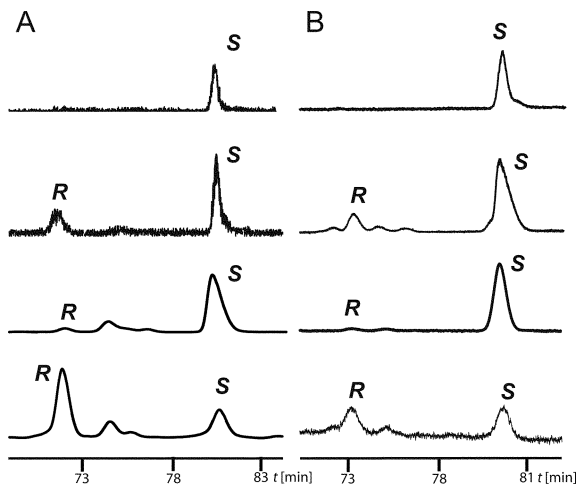


Fig. 5 GC investigation of coriolide **5** (A, ion trace 278) and of octadecatrienolide (**6**, B, ion trace *m*/*z* 276) on a chiral stationary phase (Hydrodex, 15 m, 150 °C, 0.2 °C min^{-1} to 200 °C). The following samples were analysed (from top to bottom): crude scent gland extract of *H. cydno*, co-injection scent gland extract and synthetic (*R*/*S*) mixture, synthetic (*S*) compounds, and (*R*/*S*) mixture. The analysis of *H. pachinus* showed identical results.

yield, surprisingly showing a 9 : 1 *Z* : *E* ratio in contrast to the *E*-preference often found in RCM.[17] The preference for the *Z*-isomer, identical to compound **B**, was verified by NMR analysis using NOESY spectra.

The configuration of **B** was then clarified by synthesis of pure enantiomers. Alkyne RCM[18] allowed a simple entry towards diastereomerically and enantiomerically pure products (Fig. 7), starting with 5-hexynoic acid (**14**). This acid was isomerised into 4-hexynoic acid[19] and transformed into the aldehyde **15**. Dipentylzinc was then used in an enantioselective alkylation according to Kobayashi *et al.*[20] using (1*R*,2*R*)-1,2-*N*,*N*′-bis(*p*-toluenesulfonylamino)cyclohexane as ligand for the chiral titanium catalyst. The zinc reagent always attacks from the *Re*-side when this

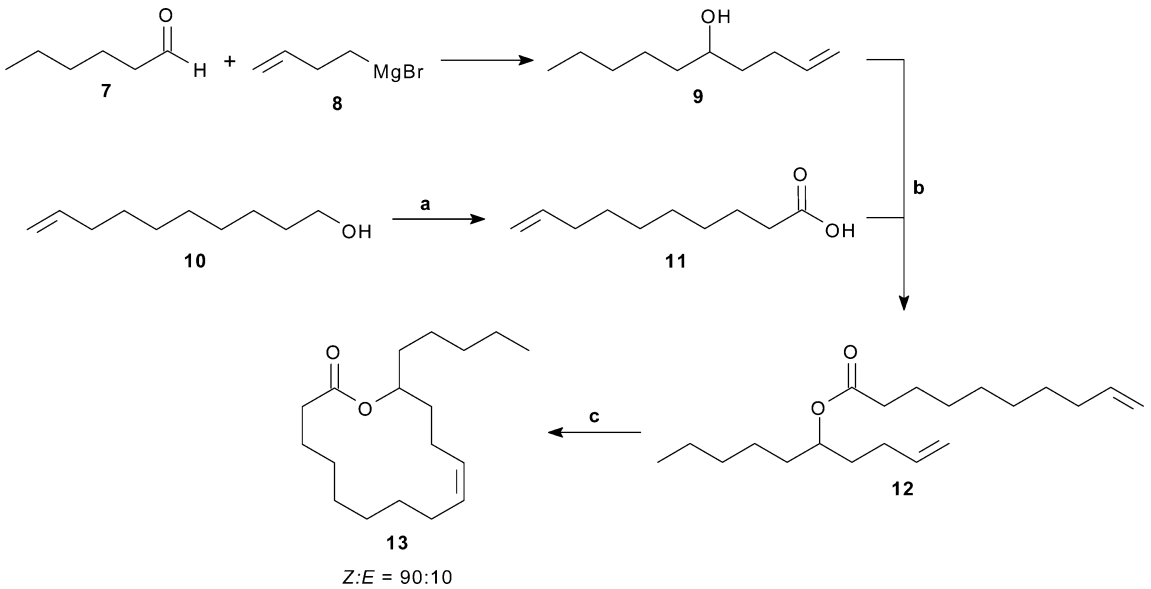


Fig. 6 Synthesis of *rac*-octadec-9-en-13-olide (**13**): a) PDC, DMF, b) $SOCl_2$, c) bis(tricyclohexylphosphane)benzylidene ruthenium(IV) dichloride, CH_2Cl_2.

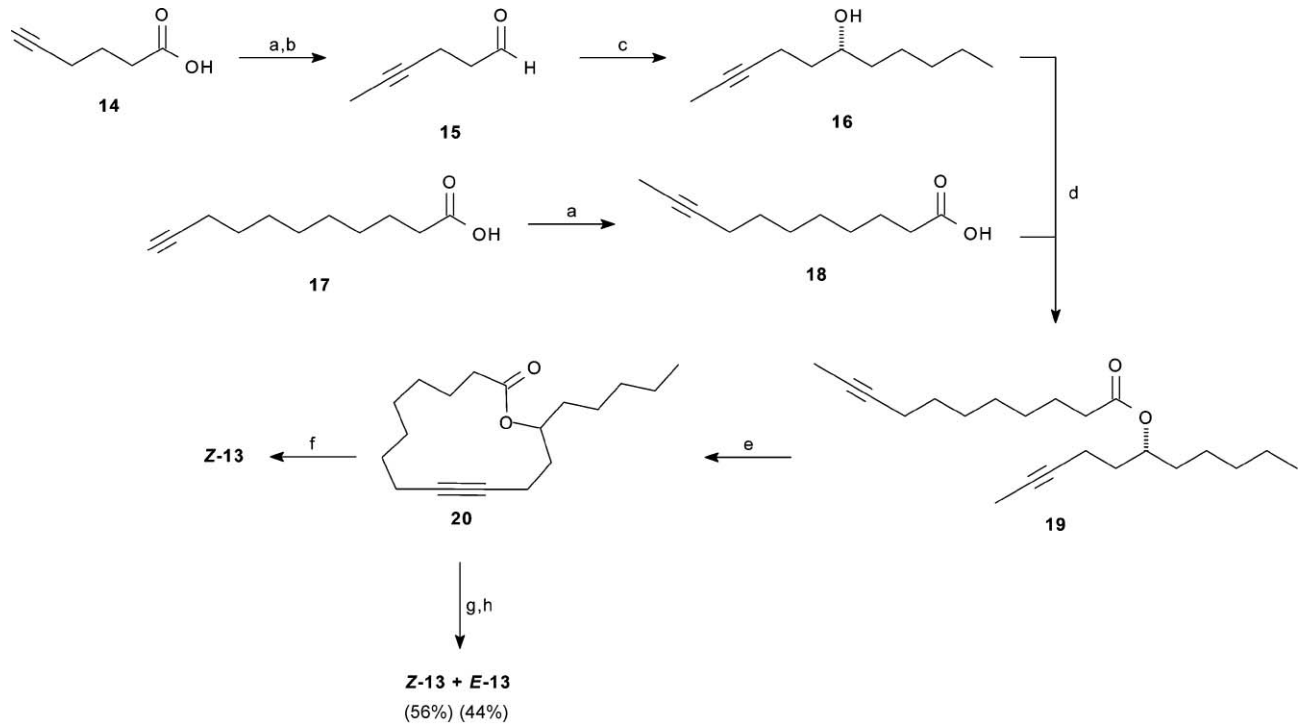


Fig. 7 Synthesis of (9*S*)-octadec-9-en-13-olide (*S*-**13**): a) KOtBu, b) DIBAL, c) $Zn(C_5H_{11})_2$, (1*R*,2*R*)-$(TsNH)_2C_6H_{10}$, $Ti(OiPr)_4$, d) $SOCl_2$, e) $Mo(CO)_6$, 4-Cl-Ph-OH, f) Lindlar cat., H_2, g) $[Cp^*Ru(MeCN)_3]PF_6$, $HSi(OEt)_3$, CH_2Cl_2, $P(CH_2OH)_3$, h) AgF, MeOH.

ligand is used, producing the alcohol (*S*)-undec-2-yn-6-ol (**16**) in moderate yield, but excellent ee (98%). Esterification with undec-9-ynoic acid (**18**), which was obtained by isomerisation from undec-10-ynoic acid (**17**),[19] gave the diynoate **19**. This precursor was submitted to alkyne RCM with $Mo(CO)_6$ and *p*-chlorophenol in chlorobenzene,[18] furnishing octadec-9-yn-13-olide (*S*-**20**) in good yield. The cyclic alkyne was finally hydrogenated under Lindlar catalysis to deliver the pure *Z*,*S*-configured macrolide *Z*,*S*-**13** with an ee of 98%. The opposite (*R*)-enantiomer was obtained by a similar sequence using the (*S*,*S*)-cyclohexanediamine derivative as ligand in the asymmetric alkylation reaction. Attempts to obtain pure (*E*)-**13** from **20** by hydrosilylation according to Fürstner *et al.*[21] were only partly successful, leading to a 44 : 56 *E* : *Z* mixture under non-optimised conditions. Nevertheless, this mixture proved to be very useful in the determination of the double bond geometry of the natural product.

Finally, the enantiomers of **13** were separated on a chiral cyclodextrin phase, showing that the natural compound possesses

exclusively *S*-configuration (see ESI†). It follows that **B** is (9*Z*,13*S*)-octadec-9-en-13-olide (*S*,*Z*-**7**).

Compound **A** was proposed to be octadec-9-en-11-olide (**25**) and synthesised *via* alkene RCM starting from octanal and vinylmagnesium bromide. The resulting dec-1-en-3-ol (**23**) was acylated with 9-decenoic acid and the resulting ester **24** submitted to RCM. In this case an 88 : 12 *E* : *Z* mixture of **25** was obtained and the structure of **A** confirmed to correspond to the *Z*-isomer. An enantioselective synthesis of **25** was then accomplished (Fig. 8). Reaction of octanoyl chloride (**21**) with ethynyltrimethylsilane gave dec-1-yn-3-one **22**. This ketone was enantioselectively reduced with $LiAlH_4$ in the presence of *N*-methyl ephedrine[22] to yield (*S*)-dec-1-yn-3-ol with moderate ee (67%). Reduction with $LiAlH_4$ furnished the decenol *S*-**23**, which was acylated as described to form *S*-**24**. Finally, RCM gave an 88 : 12-mixture of (9*E*,11*S*)-octadec-9-en-11-olide (*E*,*S*-**25**) and (9*Z*,11*S*)-octadec-9-en-11-olide (*Z*,*S*-**25**) with an ee of 67% in both cases, showing the expected *E* preference.

Attempts to synthesise pure *Z*-**25** by alkyne metathesis using the $Mo(CO)_6$ and *p*-chlorophenol system resulted in complete product decomposition. The failure may be explained by the close proximity of the ester group and the triple bond in the precursor which may lead to interaction with the catalyst with an undesired outcome.

Comparison with the natural compound **A** using gas chromatography with a 2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin phase[23] revealed that the (9*Z*,11*S*)-**25** enantiomer occurs naturally.

The three macrolides **6**, **13**, and **25** are new natural products, while *S*-coriolide **5** has been found previously in the seed oil of *Monnina emarginata*[9] and the fungus *Stagonospora*.[24] In a previous investigation[5] Meinwald *et al.* isolated **5** from male *H. pachinus* and determined its absolute configuration by optical rotatory dispersion and comparison with an authentic sample of the *S*-enantiomer obtained from *M. emarginata* as *R*-**5**. No specific rotation was reported. In contrast, the coriolides produced by both *H. pachinus* and *H. cydno* investigated by us are *S*-configured. This assignment is unambiguous, because the optical rotation of the synthesised pure compound exhibits the same sense of rotation as reported[9,12] and the soy bean lipoxygenase is known to produce *S*-configured hydroperoxides. The agreement between natural and synthesised compounds was proven by chiral GC-MS. One explanation of the discrepancy in the results obtained by Meinwald *et al.* and ours may be that the butterflies, originating from different sources, produced different enantiomers, or that the sample isolated by Meinwald *et al.* was not pure.

All natural macrolides are obviously derived from oleic, linoleic, and linolenic acids, formed by oxidation at different positions and ring closure. They all exhibit the same *S*-configuration. A minor component in the secretion is ricinollactone ((*Z*)-octadec-9-en-12-olide), showing that oxidation occurs in case of the octadecenolides at the α-, β-, and γ-positions relative to the double bond of oleic acid.

The identified macrolides might play a role in the chemical communication system of *H. cydno* and *H. pachinus*. Females contain a similar bouquet as the males, albeit in markedly lower concentration. Investigation of females before and after copulation showed that these lactones are produced by the males and transferred during copulation. Gilbert postulated that the transferred compounds generally act as antiaphrodisiacs in *Heliconius*, protecting the fertilised female from additional male courtship attempts.[2] This mechanism was recently investigated by us in more detail in *H. melpomene*.[8] In this case (*E*)-β-ocimene is transferred together with a matrix consisting of fatty acid esters. While the esters modulate the evaporation rate, ocimene, the major component of the scent glands, inhibits additional courtship attempts of males on fertilised females. It may well be that the macrolides, so far not found in other *Heliconius* sp. investigated by us or others,[3,5,8] serve this function, especially because no major component other than the macrolides was present in the glands. A defensive role, proposed for the compounds identified in the same

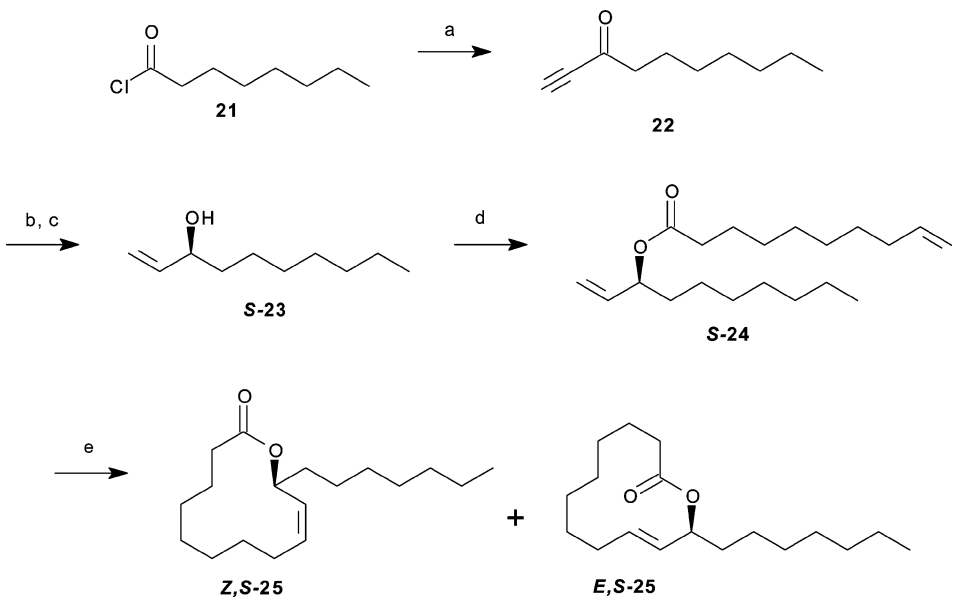


Fig. 8 Synthesis of (*S*)-octadec-9-en-11-olide (*S*-**25**): a) ethynyltrimethylsilane, $AlCl_3$, b) $LiAlH_4$, *N*-methyl ephedrine, c) $LiAlH_4$, d) dec-9-enoyl chloride, e) bis(tricyclohexylphosphane)benzylidene ruthenium(IV) dichloride.

glands of another heliconid, *Agraulis vanillae*, without mentioning the transfer mechanism,[3] might be a secondary function of these glands. Different species of *Heliconius* show largely different, species specific compositions of their scent glands, sometimes with up to 100 components of different volatility. Further work on the chemical ecology of these compounds is needed to clarify their function in the life of these impressive butterflies.

Experimental

General remarks and starting materials

Dry CH_2Cl_2 was distilled from CaH_2, diethyl ether from $LiAlH_4$, toluene from Na, and THF from K and Na. All other chemicals were commercially available (Fluka, Aldrich, Strem) and used without further treatment. All reactions were monitored by thin layer chromatography (TLC), carried out on Macherey-Nagel Polygram SIL G/UV_{254} silica plates, visualised with heat gun treatment with 10% molybdato phosphoric acid in ethanol. Column chromatography was performed with Merck silica gel 60 (70–200 mesh). GC analyses were performed with a CE instruments GC 8000 gas chromatograph equipped with a flame ionisation detector and split/splitless injection using hydrogen as carrier gas. GC-MS experiments were carried out with a Hewlett-Packard model 5973 mass selective detector connected to a Hewlett-Packard model 6890 gas chromatograph using a BPX5 fused silica capillary column (SGE, 30 m × 0.25 mm, 0.25 µm film thickness). Chiral GC and GC-MS analyses were performed with the same instruments. Chiral GC was performed on different phases: 15 m or 35 m Hydrodex-6-TBDMS (2,3-di-*O*-methyl-6-TBDMS-β-cyclodextrin, Machery & Nagel), 0.25 mm diameter, GC temperature program 150 °C, then with 0.2 °C min^{-1} to 200 °C if not stated otherwise and 15 m 2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin in 50% OV 1701 phase, 0.32 mm diameter,[23] GC temperature program 120 °C, then with 1 °C min^{-1} to 200 °C. ^1H NMR and ^{13}C NMR spectra were obtained with Bruker AC-200 and AMX-400 instruments with $CDCl_3$ as solvent. Tetramethylsilane was used as internal standard. *J* values are given in Hz. The optical rotary power was measured using a Dr. Kernchen Propol Digital Automatic polarimeter. The $[\alpha]_D^{20}$ values are given in 10^{-1}deg cm^2 g^{-1}.

Biological samples

Butterflies originating from Costa Rica were kept in a greenhouse in which *Passiflora caerulea* and *Lantana* were maintained. The butterflies had access to sugar water, and sources of pollen from *Psiguria* and *Lantana* flowers. Abdominal glands were manually exposed and directly dissected into 100 µl pentane. The samples were shipped to Braunschweig and stored at −70 °C until analysis.

Microreactions

Hydrogenation of crude scent gland extracts was carried out under Pd/C catalysis (0.5 mg) using methanol as solvent. A suspension of Pd/C in methanol was first activated by stirring for 30 min under a H_2 atmosphere. Then the scent gland extract was added and again stirred for 30 min under a H_2 atmosphere. Finally, Pd/C was removed by microfiltration over a short silica plug.

DMDS adducts were formed by addition of 50 µl DMDS and 5 µl of iodine solution (60 mg iodine in 1 ml diethyl ether) to a sample of crude natural extract. The reaction vessel was sealed and kept at 50 °C for 24 h. Then the reaction mixture was washed with saturated $Na_2S_2O_3$ solution, extracted three times with hexane, and the combined organic phases dried with NaCl. Finally, the solution was concentrated under a gentle N_2 stream and analysed by GC-MS.

General procedure A for macrolactonisation

A mixture of 2,2′-dithiopyridine (100 mg, 0.5 mmol) and triphenylphosphane (130 mg, 0.5 mmol) was dissolved in dry toluene (1 ml) under a N_2 atmosphere. Then the appropriate hydroxyacid (0.3 mmol) was added. The reaction mixture was stirred for 5 h at room temperature and then diluted with dry toluene to a 0.1 M solution. This solution was added with a syringe pump to 100 ml boiling dry toluene over a period of 15 h and heated under reflux for another 10 h. Finally, the solvent was removed *in vacuo* and the crude product purified by flash chromatography (pentane–diethyl ether, 19 : 1).

(+)-(9*Z*,11*E*,13*S*)-Octadeca-9,11-dien-13-olide *S*-5

Pure *S*-**5** (15 mg, 21%) was prepared from *S*-**3** with an ee of 97% according to general procedure A. In an identical manner *R*-enriched **3**, obtained by Mitsunobu inversion, was transformed into *R*-enriched **5**.

$[\alpha]_D^{20}$ +17.2 (*c* 0.8 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.81 (3H, t, *J* 6.9, CH_3), 1.19–1.31 (14H, m, CH_2), 1.48–1.54 (2H, m, CH_2), 1.79–1.85 (2H, m, CH_2), 2.32–2.54 (4H, m, CH_2), 5.35–5.39 (1H, m, CH), 5.45 (1H, dt, *J* 6.7 and 10.3, CH), 5.66 (1H, dd, *J* 3.7 and 15.4, CH), 5.96 (1H, t, *J* 10.8, CH), 6.43 (1H, dd, *J* 11.2 and 14.2, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 22.5 (t), 24.76 (t), 24.84 (t), 25.1 (t), 25.4 (t), 26.3 (t), 26.7 (t), 27.0 (t), 31.6 (t), 33.0 (t), 35.1 (t), 72.3 (d), 123.8 (d), 128.3 (d), 131.2 (d), 132.0 (d), 172.9 (s); EI-MS *m*/*z* (%) 43 (35), 53 (10), 55 (42), 65 (11), 67 (47), 68 (11), 69 (13), 71 (35), 77 (28), 78 (10), 79 (57), 80 (35), 81 (38), 82 (13), 83 (14), 91 (31), 93 (36), 94 (19), 95 (25), 98 (16), 99 (100), 107 (16), 108 (10), 121 (13), 135 (12), 136 (15), 151 (26), 164 (10), 179 (18), 278 (31); HR-MS $C_{18}H_{30}O_2$: calcd 278.2246, found 278.2241; chiral GC (Hydrodex, 15 m, 150 °C, 0.2 °C min^{-1} to 200 °C): rt_R = 65.74, rt_S = 71.24 min.

(+)-(9*Z*,11*E*,13*S*,15*Z*)-Octadeca-9,11,15-trien-13-olide *S*-6

Acid *S*-**4** was transformed into *S*-**6** (6 mg, 16%) according to general procedure A. In an identical manner *R*-enriched **4**, obtained by Mitsunobu inversion, was transformed into *R*-enriched **6**.

$[\alpha]_D^{20}$ +6.7 (*c* 2.0 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.96 (3H, t, *J* 7.5, CH_3, H-18), 1.25–1.62 (8H, m, CH_2), 1.86–1.92 (2H, m, CH_2, H-3), 1.99–2.13 (4H, m, CH_2, H-8 and H-17), 2.36–2.38 (2H, m, CH_2, H-14), 2.44–2.61 (2H, m, CH_2, H-2), 5.31–5.41 (1H, m, CH, H-15), 5.45–5.53 (3H, m, CH, H-13, H-16 and H-9), 5.72 (1H, dd, *J* 3.6 and 15.4, CH, H-12), 6.03 (1H, t, *J* 10.9, CH, H-10), 6.51–6.60 (1H, dd, *J* 11.3 and 15. 4, CH, H-11); δ_C (100 MHz, $CDCl_3$) 14.1 (q, C-18), 20.7 (t, C-17), 24.8 (t, C-3), 24.8 (t), 25.4 (t), 26.3 (t), 26.6 (t), 27.0 (t, C-8), 32.9 (t, C-14), 33.0 (t, C-2), 71.8 (d, C-13), 123.2 (d, C-15), 124.1 (d, C-11), 128.2 (d, C-10), 130.5

(d, C-12), 132.3 (d, C-9), 134.7 (d, C-16), 172.8 (s, C-1); EI-MS *m/z* (%) 43 (12), 55 (38), 57 (11), 65 (11), 67 (47), 69 (22), 77 (18), 79 (48), 80 (10), 81 (43), 83 (23), 91 (36), 93 (39), 95 (28), 97 (12), 105 (24),107 (19), 109 (10), 119 (16), 133 (11), 147 (33), 161 (16), 171 (23), 207 (100), 208 (16), 276 (16). HR-MS $C_{18}H_{28}O_2$: calcd 276.2089, found 276.2099; ee = 96%, chiral GC (Hydrodex, 15 m, 150 °C, 0.2 °C min^{-1} to 200 °C): rt_R 64.23, rt_S 71.05 min.

rac-(*Z*)-Octadec-9-en-13-olide *rac*-*Z*-13

A solution of **12** (28 mg, 85 μmol) in dry CH_2Cl_2 (25 ml) was treated with a catalytic amount of bis(tricyclohexylphosphane)benzylidene ruthenium(IV) dichloride (Grubbs' catalyst) under a N_2 atmosphere. The solution was heated under reflux for 36 h. Then the solvent was removed and the crude product purified by flash chromatography (pentane–diethyl ether, 19 : 1) to obtain pure *Z*-**13** (15 mg, 63%).

δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9, CH_3), 1.12–1.42 (13H, m, CH_2), 1.42–1.50 (2H, m, CH_2), 1.53–1.64 (4H, m, CH_2), 1.73–1.86 (2H, m, CH_2), 1.97–2.06 (1H, m, CH_2), 2.13–2.24 (2H, m, CH_2), 2.30–2.36 (1H, m, CH_2), 2.41–2.49 (1H, m, CH_2), 4.98–5.04 (1H, m, CH), 5.31–5.45 (2H, m, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 22.0 (t), 22.5 (t), 24.72 (t), 24.74 (t), 25.27 (t), 25.3 (t), 26.2 (t), 26.5 (t), 27.0 (t), 31.7 (t), 33.3 (t), 33.6 (t), 34.3 (t), 73.4 (d), 129.2 (d), 130.0 (d), 173.5 (s); EI-MS *m/z* (%) 43 (33), 53 (16), 54 (42), 55 (92), 56 (13), 57 (11), 67 (100), 68 (65), 69 (37), 79 (31), 80 (20), 81 (98), 82 (74), 83 (29), 93 (16), 94 (19), 95 (74), 96 (61), 97 (19), 107 (12), 108 (13), 109 (37), 110 (34), 121 (12), 122 (22), 123 (22), 124 (20), 135 (12), 136 (22), 137 (13), 138 (12), 149 (10), 150 (13), 182 (22), 196 (15), 280 (50); HR-MS $C_{18}H_{32}O_2$: calcd 280.2402, found 280.2391.

(+)-(*S*)-Undec-2-yn-6-ol 16

Pentyl bromide (10.90 ml, 13.31 g, 88 mmol) was added slowly to a mixture of magnesium (2.33 g, 97 mmol) and dry diethyl ether (100 ml) under a N_2 atmosphere. After formation of the Grignard reagent it was slowly added to a solution of anhydrous zinc chloride (5 g, 37 mmol) in dry diethyl ether (35 ml). This mixture was stirred overnight. After settling of a precipitate, the liquid phase removed using a syringe. The residue was washed three times with diethyl ether. The combined liquid phases were transferred into an distillation apparatus and distilled under a N_2 atmosphere. Dipentyl zinc (bp 100 °C, 0.1 Torr, 4.54 g, 59%) was isolated under low pressure. A solution of (1*R*,2*R*)-1,2-*N*,*N*-bis(*p*-toluolsulfonylamino)cyclohexane (31.41 mg, 0.083 mmol) in dry toluol (4 ml) and titanium tetraisopropoxylate (1.48 ml, 1.42 g, 5 mmol) was stirred under a N_2 atmosphere for 20 min at 40 °C. The mixture was cooled to −78 °C and excess dipentyl zinc (1.04 g, 5 mmol) in toluene (1 ml) and 4-hexynal (400 mg, 4.17 mmol) were added.[20] This mixture was stirred for 3 h at −78 °C and then heated to 0 °C during 5 h. A solution of HCl (2 N) was added, the mixture was washed with brine, and extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and the solvent was removed. The crude product was purified by flash chromatography (pentane–diethyl ether 5 : 1) to furnish (+)-(*S*)-**16** (301 mg, 43%).

$[\alpha]_D^{20}$ +1.9 (*c* 4.2 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.5, CH_3), 1.17–1.74 (10H, m, CH_2), 1.78 (3H, t, *J* 2.6, CH_3), 2.22–2.33 (2H, m, CH_2), 3.68–3.79 (1H, m, CH); δ_C (100 MHz, $CDCl_3$) 3.4 (q), 14.0 (q), 15.4 (t), 22.6 (t), 25.3 (t), 31.9 (t), 36.1 (t), 37.3 (t), 71.3 (d), 76.3 (s), 78.8 (s); MS *m/z* (%) 41 (93), 43 (62), 53 (38), 55 (68), 57 (13), 66 (36), 67 (52), 68 (19), 69 (38), 71 (12), 79 (38), 81 (12), 83 (23), 91 (10), 93 (26), 95 (11), 97 (100), 107 (17), 121 (18), 135 (10), 150 (7), 168 (3); HR-MS $C_{11}H_{20}O$: calcd 168.1514, found 168.1590; ee 98% (GC, 35 m Hydrodex, 100 °C, 1 °C min^{-1} to 220 °C).

(+)-(*S*)-Octadec-9-yn-13-olide *S*-20

A solution of **19** (140 mg, 0.43 mmol), *p*-chlorophenol (55 mg, 0.43 mmol), and $Mo(CO)_6$ (6 mg, 0.023 mmol) in dry 4-chlorobenzene (85 ml) was heated to reflux for 2 days under a N_2 atmosphere. The solvent was evaporated and the residue purified by flash chromatography using pentane–diethyl ether (19 : 1) to give pure **20** (74 mg, 88%).

$[\alpha]_D^{20}$ +1.2 (*c* 3.1 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9, CH_3), 1.21–1.84 (22H, m, CH_2), 2.10–2.50 (4H, m, CH_2), 4.91–4.98 (1H, m, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 14.8 (t), 18.1 (t), 18.6 (t), 22.5 (t), 23.5 (t), 24.8 (t), 25.5 (t), 26.1 (t), 26.3 (t), 26.6 (t), 31.8 (2t), 33.7 (t), 34.0 (t), 73.6 (d), 80.0 (s), 80.7 (s), 174.1 (s); EI-MS *m/z* (%) 43 (42), 53 (20), 54 (13), 55 (84), 65 (18), 66 (14), 67 (57), 68 (13), 69 (20), 77 (30), 78 (22), 79 (100), 80 (69), 81 (48), 82 (11), 83 (14), 91 (38), 92 (9), 93 (56), 94 (35), 95 (32), 96 (13), 97 (10), 105 (14), 107 (31), 108 (12), 109 (13), 119 (11), 121 (28), 122 (7), 135 (18), 136 (11), 149 (9), 150 (44), 151 (8), 167 (10), 193 (5), 207 (6), 221 (4), 236 (3), 249 (2), 278 (1); HR-MS $C_{18}H_{02}O_2$: calcd 278.2246, found 278.2209.

(9*E*,13*S*)-Octadec-9-en-13-olide 9*E*,13*S*-13

According to Fürstner *et al.*[21] 1 mol% of $[Cp^*Ru(MeCN)_3]PF_6$ (0.34 mg) was added to a solution of **20** (19 mg, 0.068 mmol) and triethoxysilane (13.3 mg, 0.081 mmol) in dry CH_2Cl_2 (2 ml) and the mixture stirred for 15 min at room temperature. Then tris(hydroxymethyl)phosphine (1.3 mg) was added and the resulting mixture was stirred for 30 min. Filtration through a short pad of silica and evaporation of the organic solvent furnished the hydrosilylated crude product. Then a solution of AgF (1 M in aq. MeOH, 0.14 ml) was added and stirring commenced for 3 h in the dark. The mixture was filtered and thoroughly washed with diethyl ether. The combined organic filtrates were concentrated and the crude product was purified by flash chromatography (pentane–diethyl ether 20 : 1) to furnish **13** (7 mg, 35%). Chiral GC (15 m Hydrodex) showed that the product was a 44 : 56 *E* : *Z* mixture with an ee of 99% in both cases.

(9*E*,13*S*)-**13**: δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.9, CH_3), 1.22–1.39 (13H, m, CH_2), 1.40–1.52 (2H, m, CH_2), 1.57–1.66 (4H, m, CH_2), 1.70–1.74 (3H, m, CH_2), 1.97–2.14 (2H, m, CH_2), 2.25–2.36 (1H, m, CH_2), 2.42–2.49 (1H, m, CH_2), 4.93–4.99 (1H, m, CH), 5.24–5.31 (1H, dt, *J* 7.3 and 15.1, CH), 5.50–5.57 (1H, dt, *J* 7.6 and 15.1, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 23.5 (t), 24.2 (t), 25.2 (t), 26.2 (t), 26.5 (t), 27.0 (t), 28.3 (t), 30.3 (t), 32.7 (t), 32.7 (t), 32.8 (t), 32.9 (t), 74.4 (d), 77.2 (t), 129.5 (d), 131.4 (d), 173.7 (s); EI-MS *m/z* (%) 41 (83), 43 (34), 54 (45), 55 (93), 67 (100), 69 (36), 79 (27), 81 (89), 95 (67), 96 (50), 109 (33), 110 (28), 122 (17), 123 (18), 136 (16), 150 (9), 164 (6), 182 (16), 196 (12), 209 (4), 223 (2), 237 (1), 262 (2), 280 (35).

(*E*)-Octadec-9-en-11-olide *E*-25

Alkene RCM was performed with **24** to furnish **25** as described above for **13** (8 mg, 32%) in a *E* : *Z* ratio of 88 : 12.

δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.2, CH_3), 0.98–2.01 (22H, m, CH_2), 2.11–2.38 (4H, m, CH_2), 5.26–5.55 (2H, m, CH), 5.65–5.83 (1H, m, CH); δ_C (100 MHz, $CDCl_3$) 14.1 (q), 22.6 (t), 24.4 (t), 25.4 (t), 25.7 (t), 26.0 (t), 26.3 (t), 28.1 (t), 29.2 (t), 29.3 (t), 31.8 (t), 32.8 (t), 33.8 (t), 35.4 (t), 74.5 (d), 130.4 (d), 137.0 (d), 174.3 (s); EI-MS *m*/*z* (%) *E*-**25**: 43 (47), 55 (98), 56 (15), 57 (35), 65 (10), 66 (10), 67 (100), 68 (39), 69 (42), 70 (11), 71 (10), 79 (36), 80 (21), 81 (81), 82 (56), 83 (39), 84 (28), 93 (21), 94 (15), 95 (54), 96 (32), 97 (35), 98 (96), 99 (10), 107 (11), 108 (15), 109 (34), 110 (37), 111 (25), 112 (11), 121 (12), 123 (14), 124 (11), 125 (17), 126 (16), 127 (29), 135 (24), 136 (15), 137 (10), 138 (15), 139 (11), 152 (15), 153 (23), 155 (12), 181 (30), 183 (10), 209 (11), 280 (15); *Z*-**25**: 43 (41), 53 (15), 54 (31), 55 (88), 56 (11), 57 (38), 67 (100), 68 (39), 69 (38), 77 (11), 79 (42), 80 (16), 81 (80), 82 (51), 83 (32), 84 (19), 91 (10), 93 (25), 94 (14), 95 (61), 96 (30), 97 (30), 98 (60), 107 (13), 109 (31), 110 (42), 111 (20), 121 (16), 123 (16), 124 (12), 125 (13), 127 (23), 135 (35), 136 (16), 138 (15), 139 (11), 152 (12), 153 (21), 181 (31), 280 (13); HR-MS $C_{18}H_{32}O_2$: calcd 280.2402, found 280.2384; chiral GC (15 m Hydrodex): $rt_{Z\text{-}25}$ 50.20, $rt_{E,R\text{-}25}$ 51.04, $rt_{E,S\text{-}25}$ 51.50 min; chiral GC (2,6-di-*O*-methyl-3-*O*-pentyl-β-cyclodextrin): $rt_{Z,R\text{-}25}$ 51.80, $rt_{Z,S\text{-}25}$ 52.15, $rt_{E\text{-}25}$ 52.65 min.

Conclusion

The major constituents of the scent glands used by the tropical longwing butterflies *Heliconius cydno* and *H. pachinus* were identified and synthesised for the first time. The synthetic compounds, accessible by short and enantioselective routes, now allow biotests, needed to understand the function of the macrolides for the complex behaviour of *Heliconius*. Given that species lacking macrolides readily hybridise with *H. cydno* and *H. pachinus*,[25] perhaps courtship and mating signals are to be discovered among the minor components of the abdominal glands and on androconial scales of the wing while the major macrolides may serve in as yet unsuspected functions such as protecting eggs from fungal disease or in protecting adults from predators.

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