Macrolides from the Scent Glands of the Tropical Butterflies *Heliconius cydno* and *Heliconius pachinus*

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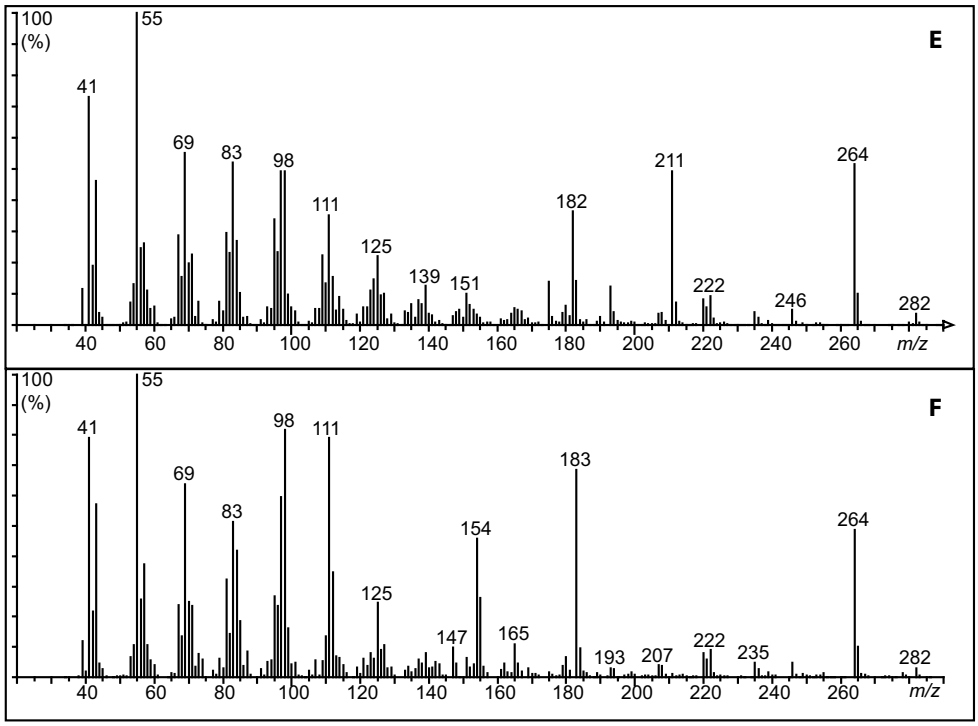

Figure S1: Mass spectra of 13-octadecanolide (E) and 11-octadecanolide (F) obtained after hydrogenantion of natural extracts of *H. pachinus* and *H. cydno*.

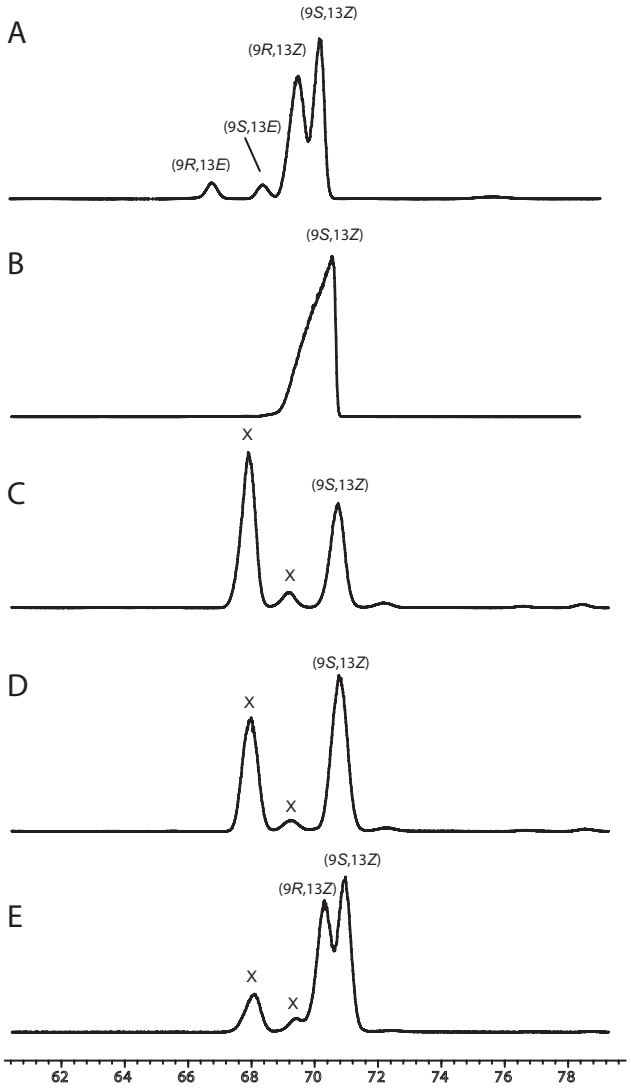

Figure S2: GC-MS analysis of octadec-9-en-13-olide (**13**) on a chiral Hydrodex phase (150°C-5 min-0.1°C/min-220°C). A: (*rac*)-**13**, B: (*S*,*Z*)-**13**, C: extract of male *Heliconius pachinus* scent gland, D: co-injection of **B** and **C**, E: co-injection of **A** and **C**. X: other components of the extract.

Experimental

General procedure B for the esterification of secondary alcohols

An appropriate acid (0.25 mmol) was treated for 1 h at 45°C with $SOCl_2$ (30 μl, 0.38 mmol) under a N_2-atmosphere. The residue was treated with the respective alcohol (0.25 mmol) dissolved in CH_2Cl_2 (2 ml). The solution was heated to reflux for 2 h. After cooling to room temperature, the mixture was treated with water (5 ml) and extracted three times with diethyl ether. The organic layer was dried ($MgSO_4$), the solvent removed, and the remaining crude product was purified by flash chromatography (pentane/diethyl ether, 19:1).

(+)-(9*Z*,11*E*,13*S*)-13-Hydroxyoctadeca-9,11-dienoic acid *S*-3

Acid *S*-**3** (110 mg, 37%) was synthesised from **1** by enzymatic oxidation according to Haffner et al.[15]

$[\alpha]_D^{20}$ +12.4 (*c* 4.3 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, *J* 6.8, CH_3), 1.26-1.64 (18H, m, CH_2), 2.14-2.20 (2H, m, CH_2), 2.33 (2H, br t, *J* 7.4, CH_2), 4.16 (1H, br q, *J* 6.4, CH), 5.40-5.46 (1H, m, CH), 5.65 (1H, dd, *J* 6.9 and 15.2, CH), 5.97 (1H, t, *J* 11.0, CH), 6.48 (1H, dd, *J* 11.1 and 15.2, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 22.5 (t), 24.6 (t), 25.0 (t), 27.5 (t), 28.8 (t), 28.85 (t), 28.88 (t), 29.3 (t), 31.7 (t), 33.9 (t), 37.2 (t), 72.8 (d), 125.7 (d), 127.8 (d), 132.6 (d), 135.7 (d), 178.9 (s).

(+)-(9*Z*,11*E*,13*S*,15*Z*)-13-Hydroxyoctadeca-9,11,15-trienoic acid *S*-4

Acid *S*-**4** (68 mg, 23%) was synthesised from **2** by enzymatic oxidation according to Haffner et al.[15]

$[\alpha]_D^{20}$ = +6.7 (*c* 1.5 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.97 (3H, t, *J* 7.5, CH_3), 1.25-1.42 (8H, m, CH_2), 1.56-1.65 (2H, m, CH_2), 2.04-2.11 (2H, m, CH_2), 2.17 (2H, q, *J* 7.3, CH_2), 2.29-2.40 (4H, m, CH_2), 4.22 (q, 1H, *J* 6.3, CH), 5.28-5.47 (2H, m, CH), 5.54-5.58 (1H, m,

CH), 5.69 (1H, dd, *J* 6.3 and 15.2, CH), 5.97 (1H, t, *J* 11.0 Hz, CH), 6.52 (1H, dd, *J* 11.1 Hz and 15.5, CH); δ_c (100 MHz, $CDCl_3$) 14.2 (q), 20.7 (t), 24.6 (t), 27.6 (t), 28.8 (t), 28.9 (t), 29.3 (t), 29.9 (t), 33.9 (t), 35.2 (t), 72.1 (d), 123.7 (d), 125.9 (d), 127.8 (d), 132.9 (d), 134.9 (d), 135.3 (d), 179.1 (s).

Mitsunobu-Inversion of hydroxyacids 3 and 4

A solution of ***S*-3** or ***S*-4** (28 mg, 0.09 mmol), triphenylphosphane (39 mg, 0.14 mmol) and benzoic acid (17 mg, 0.14 mmol) in dry THF (3 ml) was treated with diethyl azodicarboxylate (25 μl, 0.14 mmol) under a N_2-atmosphere. The solvent was evaporated after stirring for 10 min at room temperature and methanol (2 ml) and NaH (5 mg, 0.20 mmol) were added to the residue. The reaction mixture was stirred overnight at room temperature and acidified with 1N HCl. The aqueous phase was extracted three times with diethyl ether, the combined organic phases dried with $MgSO_4$, and the solvent removed. The crude products were purified by flash chromatography using pentane/diethyl ether (1:1) as solvent to furnish pale yellow oils (72%/76%). Analysis of the products of the macrolactonisation showed that the inversion furnished non-racemic mixtures in which the (*R*)-enantiomers predominated.

Dec-1-en-5-ol 9

A solution of 4-bromo-1-butene (150 mg, 1.1 mmol) in dry THF (1 ml) was slowly added under N_2-atmosphere to Mg (27 mg, 1.1 mmol) in dry THF (0.5 ml). The reaction was started with 2 drops of 1,2-dibromoethane and the remaining bromobutene solution was added. After the addition, the mixture was treated with a solution of hexanal (90 mg, 0.9 mmol) in dry THF (5 ml) and heated for 30 min to reflux. The reaction was quenched with saturated NH_4Cl (20 ml) 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 give pure **9** (96 mg, 62%).

Spectroscopic NMR data were identical to those published earlier.[26] EI-MS *m/z* (%) 43 (44), 44 (10), 54 (24), 55 (100), 56 (25), 57 (41), 58 (16), 67 (67), 68 (16), 71 (12), 81 (18), 82 (14), 83 (71), 85 (31), 96 (18), 101 (10), 110 (14).

1-Decen-5-yl 9-decenoate 12

Compound **12** (60 mg, 75%) was prepared according to the general procedure A from **9** and 9-decenoic acid (**11**). The latter was prepared by Corey-Schmidt oxidation[27] of **10**.

δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 6.7, CH_3), 1.23-1.69 (m, 22H, CH_2), 2.01-2.08 (2H, m, CH_2), 2.27 (2H, t, *J* 7.6, CH_2), 4.05 (1H, t, *J* 6.8, CH), 4.88-5.02 (4H, m, CH_2), 5.75-5.84 (2H, m, CH); δ_C (100 MHz, $CDCl_3$) 14.0 (q), 22.5 (t), 24.9 (t), 25.1 (t), 28.8 (t), 28.9 (t), 29.08 (t), 29.11 (t), 29.6 (t), 31.7 (t), 33.4 (t), 33.7 (t), 34.1 (t), 34.7 (t), 73.5 (d), 114.2 (t), 114.8 (t), 138.0 (d), 139.1 (d), 173.6 (s); EI-MS *m/z* (%) 43 (24), 54 (32), 55 (100), 56 (13), 57 (14), 67 (50), 68 (31), 69 (57), 79 (12), 81 (40), 82 (35), 83 (57), 84 (14), 93 (15), 95 (22), 96 (53), 97 (29), 107 (10), 109 (21), 110 (37), 111 (13), 135 (81), 136 (10), 138 (23), 152 (14), 153 (19).

4-Hexynoic acid

The isomerisation of the triple bond of 5-hexynoic acid (3.8 g, 77%) was performed according to the procedure described for **18**.

The ^{1}H NMR and MS data were identical to those reported earlier.[28] δ_C (100 MHz, $CDCl_3$) 3.4 (q), 14.4 (t), 33.8 (t), 76.6 (s), 76.9 (s), 178.5 (s).

4-Hexynal 15

4-Hexynoic acid (3.84 g, 34.29 mmol) was reduced with $LiAlH_4$ (2.41 g, 63.3 mmol) in dry diethyl ether (120 ml) using standard procedures. Purification by flash chromatography (pentane/diethyl ether 4:1) furnished a colourless liquid (4 g, 65%). The spectroscopic data were identical to those reported earlier.[28, 29] The thus formed 4-hexynol (1 g, 10.20 mmol) in

15 ml dry dichloromethane was slowly added to pyridinium dichromate (7.69 g, 20.45 mmol) dissolved in dry CH_2Cl_2 (35 ml) under a N_2-atmosphere. After stirring for 5 h, the residue was filtered through a small silica column. The solvent was removed and the crude product was purified by flash chromatography (pentane/diethyl ether 5:1) to furnish 4-hexynal (1.1 g, 57%). δ_H (400 MHz, $CDCl_3$) 1.76 (3H, t, *J* 2.6, CH_3), 2.42-2.50 (2H, m, CH_2), 2.59-2.65 (2H, m, CH_2), 9.79 (1H, t, *J* 1.4, CHO); δ_C (100 MHz, $CDCl_3$) 3.4 (q), 12.1 (t), 42.9 (t), 76.8 (s), 76.9 (s), 201.1 (d); EI-MS *m/z* (%) 39 (60), 41 (63), 51 (20), 53 (56), 65 (23), 67 (46), 68 (35), 81 (100), 95 (20), 96 (2).

9-Undecynoic acid 18

10-Undecynoic acid (**17**, 180 mg, 1mmol) was dissolved in DMSO (2 ml) and potassium *tert*-butanolate (200 mg, 2 mmol) was added. The mixture was stirred for 10 min at 75°C. Then it was acidified with 1N HCl-solution, extracted three times with diethyl ether, the combined organic extract were dried with $MgSO_4$, and the solvent removed to furnish **18** (120 mg, 67%) sufficiently pure for the next step. The ^{1}H NMR and MS data were identical to those reported earlier.[30]

(*S*)-2-Undecyn-6-yl 9-undecynoate 19.

Ester *S*-**19** (146 mg, 59%) was prepared according to the general procedure B from **16** and **18**. $[\alpha]_D^{20}$ +3.4 (*c* 3.8 in diethyl ether); δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 7.5, CH3), 1.73-1.21 (20H, m, CH_2), 1.76 (3H, t, *J* 2.5, CH_3), 1.78 (3H, t, *J* 2.5, CH_3), 2.07-2.20 (4H, m, CH_2), 2.28 (2H, t, *J* 7.4, CH_2), 4.94 (1H, quin, *J* 6.2, CH); δ_C (100 MHz, $CDCl_3$) 3.4 (2q), 14.0 (q), 15.1 (t), 18.7 (t), 22.5 (t), 24.8 (t), 25.0 (t), 28.7 (t), 28.8 (t), 29.0 (2t), 31.7 (t), 33.5 (t), 33.9 (t), 34.6 (t), 73.1 (d), 75.4 (s), 75.8 (s), 78.2 (s), 79.3 (s), 173.5 (s); EI-MS *m/z* (%) 43 (33), 53 (41), 54 (17), 65 (14), 66 (53), 67 (74), 68 (38), 69 (31), 77 (21), 79 (66), 80 (17), 81 (81), 82 (11), 83 (13), 91 (23), 93 (66), 94 (34), 95 (100), 96 (15), 97 (12), 105 (15), 107 (40), 108

(16), 109 (24), 119 (16), 121 (44), 122 (12), 123 (17), 133 (10), 135 (19), 147 (13), 150 (14), 151 (8), 168 (8), 195 (22), 210 (8), 261 (5), 275 (5), 289 (2), 303 (1), 317 (2), 332 (0.2).

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

A catalytic amount of Lindlar catalyst was activated by stirring for 30 min in dry methanol (2 ml) under a H_2-atmosphere. Then a solution of **20** (20 mg, 0.07 mmol) in dry methanol (0.5 ml) was added and stirred for another 30 min. The reaction mixture was filtered over a short silica plug and the solvent was removed to furnish pure *Z*-**13** (14 mg, 72%). Analytical data see *rac*-*Z*-**13**.

Dec-1-en-3-ol 23

Several drops of a vinyl bromide solution (5 ml, 5 mmol, 1M in THF) were added to Mg turnings (12 mg, 5 mmol) in dry THF (0.5 ml) under a N_2-atmosphere. The reaction was started with two drops of 1,2-dibromoethane and the remaining vinyl bromide solution slowly added. Then a solution of freshly distilled octanal (0.75 ml) in dry THF (3 ml) was added and the mixture heated under reflux for 30 min. The solution was treated with saturated NH_4Cl (5 ml) and the aqueous phase extracted three times with diethyl ether. The combined organic phases were dried with $MgSO_4$ and the solvent evaporated. The crude product was purified by flash chromatography using pentane/diethyl ether (5:1) as solvent. (460 mg, 61%).
The ^{1}H NMR[31] and MS data[32] were identical to those reported earlier.

1-Decen-3-yl dec-9-enoate 24

Ester **24** (90 mg, 46%) was prepared according to the general procedure from **23** and 9-decenoic acid.

δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J* 7.0, CH_3), 1.25-1.63 (22H, m, CH_2), 2.04 (2H, q, *J* 6.8, CH_2), 2.29 (2H, t, *J* 6., $CH_2$8), 4.92-5.24 (5H, m, CH_2), 5.73-5.86 (2H, m, CH); δ_C (100 MHz,

$CDCl_3$) 14.1 (q), 22.6 (t), 25.0 (t), 25.1 (t), 28.8 (t), 28.9 (t), 29.07 (t), 29.09 (2t), 29.2 (t), 29.3 (t), 31.8 (t), 33.7 (t), 34.2 (t), 74.5 (d), 114.2 (t), 116.3 (t), 136.8 (d), 139.1 (t), 173.1 (s); EI-MS *m/z* (%) 43 (29), 54 (27), 55 (86), 56 (11), 57 (23), 67 (46), 68 (20), 69 (59), 79 (12), 81 (27), 82 (16), 83 (60), 84 (12), 93 (16), 95 (15), 96 (17), 97 (25), 98 (15), 107 (13), 109 (18), 110 (15), 127 (22), 135 (100), 136 (12), 138 (10), 153 (25), 156 (15).

Dec-1-yn-3-one 22

Ketone **22** was prepared in 77% yield according to a published procedure.[33] The NMR data were identical to those reported earlier.[34]

(-)-(*S*)-Dec-1-yn-3-ol.

Under a N_2-atmosphere a solution of (+)-*N*-methyl ephedrine (880 mg, 5 mmol) in dry diethyl ether (4 ml) was added to $LiAlH_4$ (190 mg, 5 mmol) in dry diethyl ether (1.5 ml) over a period of 30 min. After stirring for 1 hour, the reaction mixture was cooled to -15°C and **22** (250 mg, 1.7 mmol) was added dropwise over a period of 1 hour. The solution was stirred for 15 min and brine (10 ml) was added at 0°C. The organic phase was washed twice with 1N HCl to separate *N*-methyl ephedrine. Then the organic phase was treated twice with 1N NaOH, dried with $MgSO_4$, and the solvent was removed. The crude product was purified by flash chromatography (pentane/diethyl ether 2:1) to obtain pure (*S*)-dec-1-yn-3-ol (225 mg, 86%). The ee of 67 % was determined by GC on a chiral stationary phase (15 m Hydrodex, 50°C-1°C/min-180°C).

The NMR[34] and MS[32] data were identical to those reported earlier. $[\alpha]_D^{20}$ - 2.5 (*c* 7.0 in diethyl ether); chiral GC (min): rt_S= 58.6, rt_R= 60.0.

(+)-(S)-Dec-1-en-3-ol *S*-23

According to Vigneron and Bloy,[22] A solution of (*S*)-dec-1-yn-3-ol (120 mg, 0.8 mmol) in dry THF (2 ml) was added to $LiAlH_4$ (30 mg, 0.6 mmol) and dry THF (4 ml) under a N_2-atmosphere. After stirring for 3 h under reflux the mixture was carefully treated with water (5 ml) and washed with 1N NaOH. The aqueous phase was extracted three times with diethyl ether and the combined organic phases were dried with $MgSO_4$. After removal of the solvent the product was obtained sufficiently pure for the next step. Only small amounts of the educt and decan-3-ol were present as impurities.

$[\alpha]_D^{20}$ +1.2 (*c* 3 in diethyl ether).

(-)-(*S*)-Dec-1-en-3-yl 9-decenoate *S*-24

Ester *S*-**24** (74 mg, 62%) was prepared according to the general procedure B from *S*-**23** and 9-decenoic acid.

$[\alpha]_D^{20}$ -1.3 (*c* 2.3 in diethyl ether).

(+)-(9*E*,13*S*)-Octadec-9-en-11-olide *E*,*S*-25

The reaction was performed with *S*-**24** as described above for **13** to furnish *S*-**25** (34 mg, 41%) in a *E*/*Z* ratio of 88:12 and an ee of 67 %.

$[\alpha]_D^{20}$ +0.3 (*c* 3.6 in diethyl ether).

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