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Penta-deuterated acid precursors in the pheromone biosynthesis of the Egyptian armyworm, *Spodoptera littoralis*

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Synthesis of penta-deuterated intermediate precursors d_5(E)-11-14:Acid (7), d_5(Z)-11-14:Acid (10) and $d_5$14:Acid (12) of the pheromone of the Egyptian armyworm *Spodoptera littoralis* has been accomplished by a very convenient route starting from the commercially available 9-dodecyn-1-ol. The processes occur with a high to excellent chemical and stereochemical yields and the compounds have been successfully used in the confirmation of the pheromone composition and biosynthesis of our strain.

Keywords: deuterium-labelled fatty acids synthesis; labelled pheromone precursors; pheromone biosynthesis

Introduction

The Egyptian armyworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a widely distributed pest of cotton plants and vegetable crops in Europe and Africa. Female pheromone composition appears to be highly dependent on the origin of the strain and changes may occur with time within the same location.[1] In our previous work we analysed the pheromone composition of our current Spanish colony as a mixture of (*Z*,*E*)-9,11-tetradecadienyl acetate ((*Z*,*E*)-9,11-14:OAc) (**I**), (*Z*)-9-tetradecenyl acetate ((*Z*)-9-14:OAc) (**II**), (*E*)-11-tetradecenyl acetate ((*E*)-11-14:OAc) (**III**), tetradecyl acetate (14:OAc) (**IV**), (*Z*)-11-tetradecenyl acetate ((*Z*)-11-14:OAc) (**V**) and (*E*,*E*)-10,12-tetradecadienyl acetate ((*E*,*E*)-10,12-14:OAc) (**VI**) in 57:11:11:1:7:14 ratio[1]. In this paper, we report a full account of the synthesis of the intermediate labelled precursors $d_5$14:Acid, d_5(*E*)-11-14:Acid and d_5(*Z*)-11-14:Acid in the pheromone biosynthesis of the armyworm as well as a re-study of the biosynthetic steps leading to the pheromone compounds cited above[2]. The prepared penta-deuterated fatty acids and methyl esters present GC retention times clearly different than those of the unlabelled materials improving the difference between the latter chemicals with the corresponding tri-deuterated compounds.[2]

Results and discussion

The synthetic scheme for preparation of d_5(*E*)-11-14:Acid (**7**), d_5(*Z*)-11-14:Acid (**10**) and $d_5$14:Acid (**12**) is shown in Scheme 1. Preparation of the key intermediate **4** was obtained by triple bond migration[3] of the commercially available 9-dodecyn-1-ol (**1**) (Alfa Aesar GmbH, Karlsruhe, Germany) to obtain alcohol **2** followed by MOM-protection and alkylation with per-deuterated iodoethane. Reduction of **4** with sodium/ammonia and removal of the protecting group afforded labelled alcohol **6**, stereochemically pure by ^{13}C NMR, which was oxidized with Jones reagent to provide d_5(*E*)-11-14:Acid (**7**) in 63.5% overall yield from **1**. On the other hand, hydrolysis of the MOM group of **4** yielded alcohol **8** that was subjected to partial hydrogenation with P-2 Ni in ethanol[4] to furnish alcohol **9**, stereochemically pure by ^{13}C NMR, followed by Jones oxidation to give d_5(*Z*)-11-14:Acid (**10**) in 67.7% overall yield from **1**. Complete hydrogenation of **8** with Pd/C provided labelled alcohol **11**, which after oxidation provided $d_5$14:Acid (**12**) in 62.2% overall yield from the starting alcohol **1**.

With the labelled pheromone precursors in hand, study of the pheromone biosynthesis of our strain was conducted by application of the labelled fatty acids to the female gland. About 2 h after the treatment, pheromone glands were excised and extracted in hexane. As shown in Figure 1, application of $d_3$16:Acid and monitoring of the M+3 peaks resulted in labelling of all acetates **I**–**VI** present in the pheromone gland (compounds **IV** and **V** were inseparable under our GC conditions) showing that palmitic acid is the biosynthetic precursor of all pheromone components and intermediates (see below). Application of $d_5$14:Acid resulted in label incorporation on acetates **I**, **III**, **V** and **VI** but not **II**[2]. Application of d_5(*E*)-11-14:Acid labelled acetate **III** and the major component **I** whereas treatment with d_5(*Z*)-11-14:Acid incorporated labelling into acetate **V** and the minor diene acetate **VI**, but not into the main acetate **I** (Figure 1).

On the other hand, alkaline methanolysis of the glandular tissues followed by hexane extraction and analysis by GC-MS allowed us to identify the corresponding labelled fatty acid

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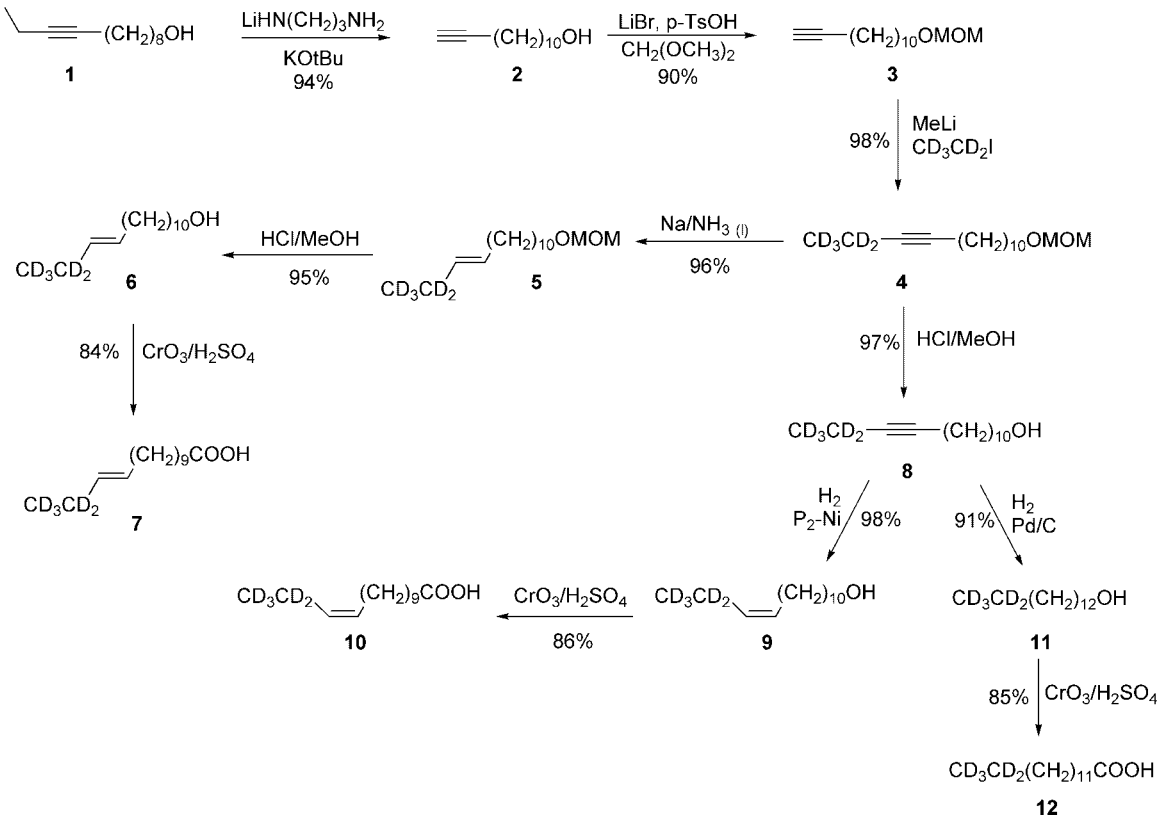

Scheme 1.

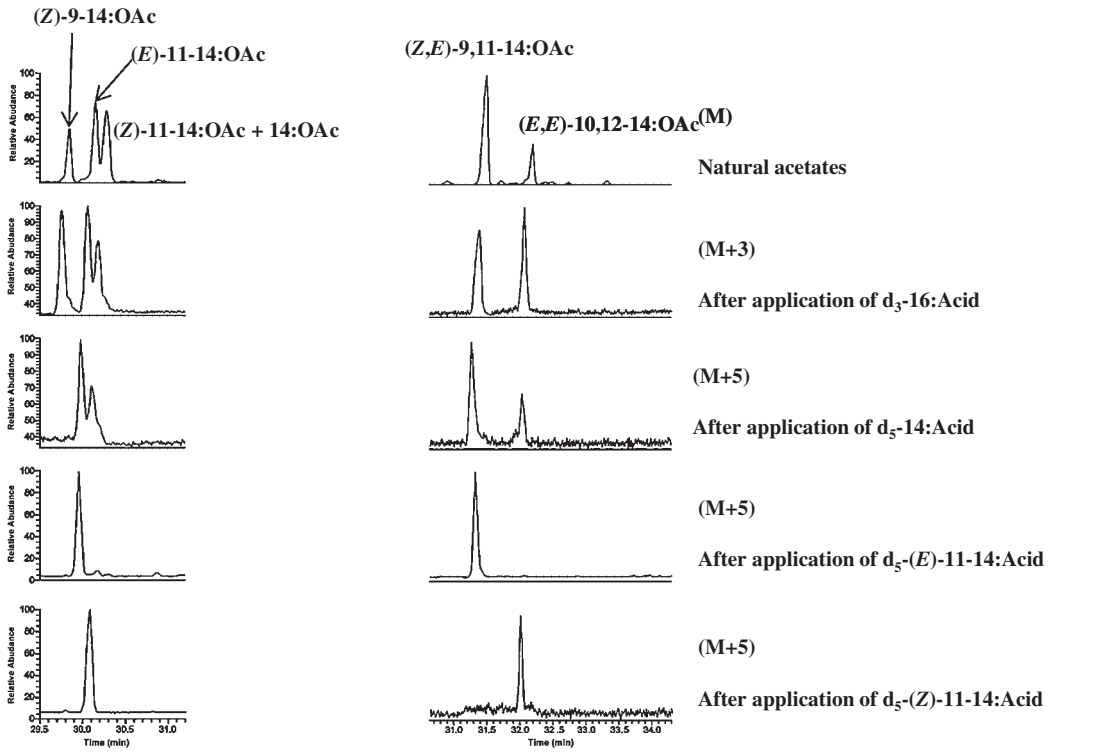

Figure 1. Partial GC-MS chromatograms of pheromone gland extracts obtained from *S. littoralis* after application of the deuterium-labelled fatty acids. Ions monitored by selected ion monitoring (SIM) correspond to the molecular weight M (unlabelled), M+3 and M+5 (ions at *m/z* 252, 255 and 257, respectively) for acetates **I** and **VI** and to loss of acetic acid M-60 (unlabelled), M-60+3, and M-60+5 (ions at *m/z* 194,196; 197, 199; and 199, 201, respectively) for acetates **II**, **III**, **IV** and **V**. The upper trace corresponds to the natural pheromone composition.

methyl esters as intermediates of the biosynthetic pathway. Now, again, application of $d_3$16:Acid resulted in labelling of all expected methyl esters (ions at *m/z* M+3 with regard to the synthetic esters) indicating that palmitic acid is the starting fatty acid in the biosynthetic pathway (Figure 2). Application of $d_5$14:Acid, d_5(*E*)-11-14:Acid and d_5(*Z*)-11-14:Acid confirmed the presence of the intermediate fatty acid methyl esters (ions at *m/z* M+5 with respect to the synthetic unlabelled methyl esters) (Figure 2) of the proposed biosynthesis[1,2] (Figure 3). Here, again, the presence of the minor diene ester (Z,E)-9,12-14:Me was not detected confirming the absence of the corresponding acetate in the pheromone composition of our strain. This unconjugated diene (Z,E)-9,12-14:OAc is one of the pheromone components in Kenyan[5] and Israeli[6] populations of *S. littoralis*, and its biosynthesis has been demonstrated to involve a desaturation step by a unique Δ-12 desaturase.[7]

Experimental

General

All reactions involving air or moisture sensitive materials were carried out under Ar. All solvents were dried and distilled

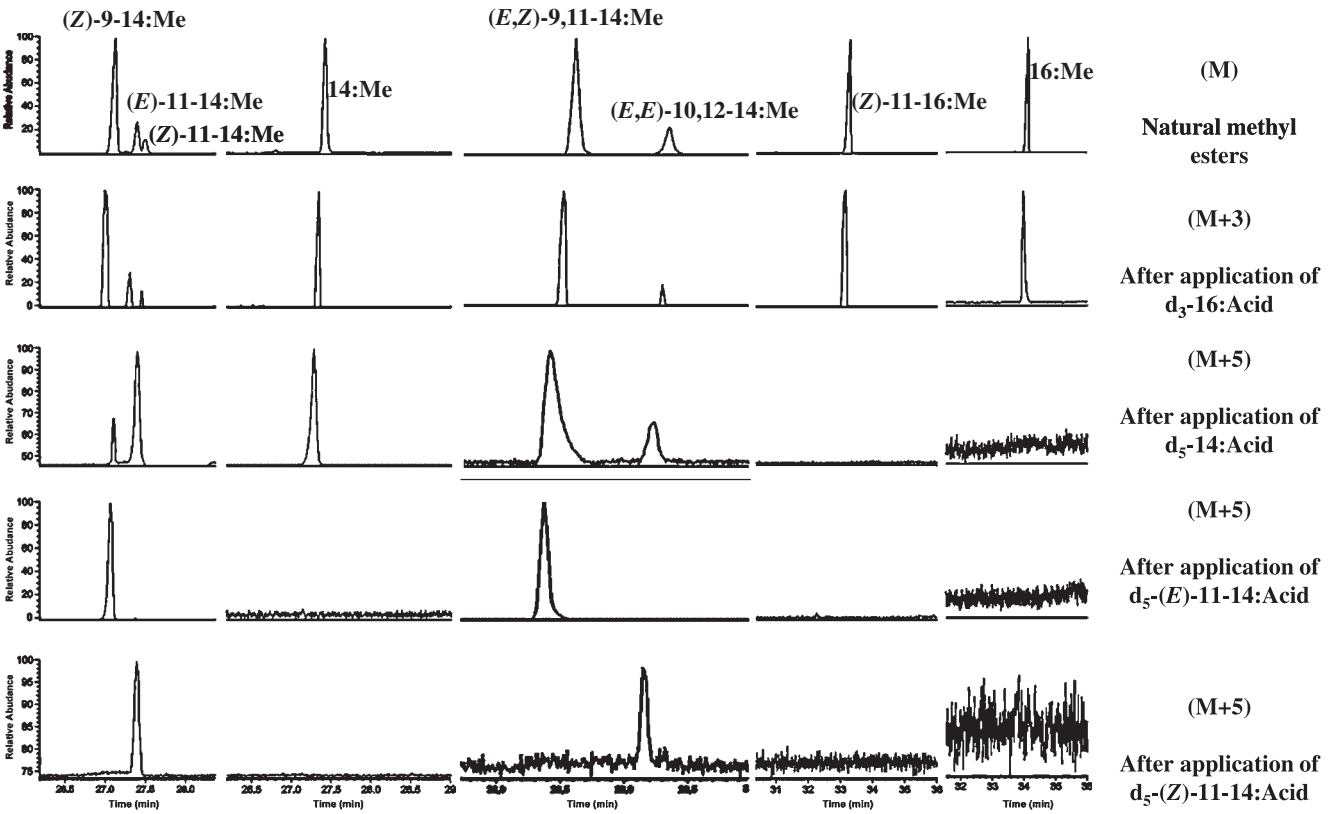

Figure 2. Partial GC-MS chromatograms of alkaline methanolysis of pheromone gland extracts of *S. littoralis* after application of the deuterium-labelled fatty acids. Ions monitored by selected ion monitoring (SIM) correspond to the molecular weight M (unlabelled) (ions at *m/z* 270 for 16:Me, 268 for (*Z*)-11-16:Me, 242 for (*E,Z*)-9,11-14:Me and (*E,E*)-10,12-14:Me, 240 for 14:Me and 238 for (*Z*)-9-14:Me, (*E*)-11-14:Me and (*Z*)-11-14:Me) of the synthetic methyl esters, and to M+3 (273, 271, 245, 243, and 241) and M+5 (275, 273, 247, 245, and 243) corresponding to labelling with 3 and 5 deuterium atoms, respectively. The upper trace corresponds to a mixture of natural unlabelled fatty acid methyl esters.

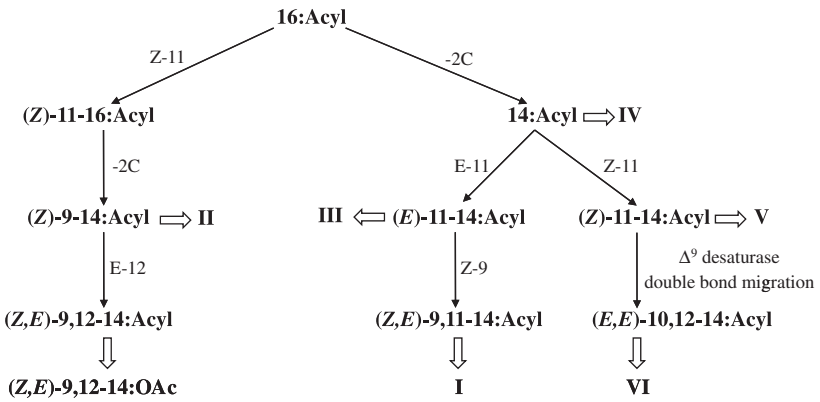

Figure 3. Biosynthetic pathways of the sex pheromone of *S. littoralis* (adapted from Reference[1]).

according to standard procedures. The purity and location of deuterium label in all substrates were confirmed by elemental analysis, MS and ^{13}C NMR. IR spectra were recorded on a Bomen MB-120 or on a Nicolet Avatar 360 FT–IR spectrometer. NMR spectra were recorded at 300 or 500 MHz for ^{1}H and 100 MHz for ^{13}C on a Varian Unity 300, Varian Mercury 400 or Varian Inova 500 MHz spectrometer. Mass spectra (MS) were obtained on a Fisons MD 800 instrument. Elemental analyses were determined on a Carlo Erba-1106, Carlo Erba EA-1108 or Perkin Elmer CHN 2400 analyzer. Melting points were determined on a Koffler apparatus.

Biosynthetic analysis

S. littoralis females were anesthetized with CO_2 and fixed with a net so that pheromone glands were forcibly extruded. Labelled compounds were topically applied to the gland surface and a dose of 1 μg of each labelled intermediate in 0.1 μl of DMSO was administered per insect except [16,16,16-$^{2}H_3$]-hexadecanoic acid of which 5 μg were applied.

Insects were treated before the onset of their second scotophase, released from the netting 30 min later, and returned to their regular photoperiod conditions. The pheromone glands (groups of 15) were excised and extracted with hexane 2 h after treatment to analyse the pheromone acetates. For the fatty acyl intermediates, glands were subjected to alkaline methanolysis and the extracts were stored at −20°C until GC-MS analysis.

11-Dodecyn-1-ol (2)[3]

To a three-necked round-bottomed flask containing lithium (1.22 g, 175.8 mmol), previously washed with hexane, was added propane-1,3-diamine (84 ml, 1006.23 mmol). The mixture was stirred at room temperature for 30 min (blue colour) and heated at 75°C until a white suspension of lithium amide was formed. The reaction mixture was cooled to room temperature and K^{t}BuO (13.76 g, 122.62 mmol) was added (yellow solution). Stirring was continued for 20 min and then 9-dodecyn-1-ol (5.00 g, 27.43 mmol) in propane-1,3-diamine (23 ml, 275.51 mmol) was added dropwise via cannula. The reddish-brown mixture was stirred for 2 h and poured onto ice/water, extracted with hexane and washed with $NaHSO_4$, brine and dried ($MgSO_4$). Purification by column chromatography on silica gel eluting with hexane:ether 3:2 furnished 4.69 g (94%) of alcohol **2** as a white waxy solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (t, J = 6.6 Hz, 2H); 2.17 (td, J_1 = 6.9 Hz, J_2 = 2.7 Hz, 2H); 1.93 (t, J = 2.7 Hz, 1H); 1.47–1.58 (m, 4H); 1.28–1.40 (bs, 12H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 84.77 (C); 68.0 (CH); 63.0 (CH_2); 32.7 (CH_2); 29.5 (CH_2); 29.4 (CH_2); 29.3 (CH_2); 29.0 (CH_2); 28.7 (CH_2); 28.4 (CH_2); 25.7 (CH_2); 18.4 (CH_2) ppm. IR (film) υ: 3311, 2929, 2857, 1456, 1046 cm^{-1}. MS (EI) *m/z* (%): 149 (9); 135 (49); 121 (65); 107 (73); 95 (84); 81 (100); 67 (96); 55 (86); 41 (90).

1-Methoxymethyloxy-11-dodecyne (3)

A mixture of 11-dodecyn-1-ol (4.49 g, 24.63 mmol), LiBr (0.45 g, 5.20 mmol) and *p*-toluenesulfonic acid (0.39 g, 2.03 mmol) was stirred at rt in dimethoxymethane (50 ml, 565 mmol) for 4 h. Brine was then added and the mixture was extracted with hexane. The combined organic extracts were dried over $MgSO_4$ and the solvent removed under vacuum. After purification on column chromatography over silica gel eluting with hexane:Et_2O 9:1, compound **3** (5.03 g, 90%) was obtained as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.59 (s, 2H); 3.49 (t, J = 6.6 Hz, 2H); 3.33 (s, 3H); 2.15 (td, J_1 = 6.9 Hz, J_2 = 2.7 Hz, 2H); 1.91 (t, J = 2.7 Hz, 1H); 1.45–1.61 (m, 4H); 1.26–1.38 (bs, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 96.3 (CH_2); 84.7 (C); 68.0 (CH); 67.8 (CH_2); 55.0 (CH_3); 29.7 (CH_2); 29.5 (CH_2); 29.4 (CH_2); 29.0 (CH_2); 28.7 (CH_2); 28.4 (CH_2); 26.1 (CH_2); 18.3 (CH_2) ppm. IR (film) υ: 3290, 2931, 2853, 1458, 1043, 913, 632 cm^{-1}. MS (EI) *m/z* (%): 225 [(M-1)$^{+}$, 5]; 147 (29); 135 (41); 121 (45); 107 (47); 95 (57); 75 (55); 67 (58); 55 (53); 45 (100).

[13,13,14,14,14-$^{2}H_5$]-1-Methoxymethyloxy-11-tetradecyne (4)[8]

To a solution of **3** (1.28 g, 5.65 mmol) in anh. THF (13 ml) were added 4.5 ml (6.78 mmol) of 1.5 M MeLi at 0°C under Ar. Stirring was maintained at 0°C for 30 min and then a solution of per-deuterated ethyl iodide in anh. HMPA (5.9 ml, 33.95 mmol) was added. The mixture was stirred at rt overnight and quenched with NH_4Cl sat. soln. After extraction with hexane, the organic layer was washed with 1N HCl, dried over $MgSO_4$ and evaporated under reduced pressure. The crude was purified by column chromatography on silica gel eluting with hexane:Et_2O 95:5 to afford 1.44 g (98%) of compound **4** as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.60 (s, 2H); 3.50 (t, J = 6.6 Hz, 2H); 3.34 (s, 3H); 2.11 (t, J = 6.6 Hz, 2H); 1.40–1.62 (m, 4H); 1.26–1.36 (bs, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 96.3 (CH_2); 81.4 (C); 79.5 (C); 67.8 (CH_2); 55.0 (CH_3); 29.7 (CH_2); 29.5 (CH_2); 29.4 (CH_2); 29.3 (CH_2); 29.1 (CH_2); 28.8 (CH_2); 13.3 (hept., J = 19.6 Hz, CD_3); 11.5 (quint., J = 19.6 Hz, CD_2) ppm. IR (film) υ: 2926, 2858, 1455, 1145, 1116, 1043, 919 cm^{-1}. MS (EI) *m/z* (%): 259 [(M)$^{+}$, 1]; 225 (11); 208 (20); 193 (27); 140 (33); 114 (47); 100 (47); 86 (57); 81 (50); 55 (46); 45 (100).

[13,13,14,14,14-$^{2}H_5$]-(*E*)-1-Methoxymethyloxy-11-tetradecene (5)[9]

In a three-necked flask under Ar ammonia was condensed (200 ml) at −35°C. Sodium (0.31 g, 13.40 mmol) was added in small pieces followed by a solution of 0.70 g (2.70 mmol) of **4** in anh. Et_2O (2 ml) and the reaction mixture stirred at −35°C until the reaction was complete (5 h). Then, NH_4Cl (s) was added and the ammonia was allowed to evaporate by warming to rt. Water was added and the organic material extracted with hexane. The combined organic layers were washed with water and dried ($MgSO_4$). Removal of the solvent yielded 0.68 g (96%) of stereomerically pure **5**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.40 (m, 2H); 4.62 (s, 2H); 3.51 (t, J = 7 Hz, 2H); 3.36 (s, 3H); 1.96 (m, 2H); 1.59 (m, 2H); 1.26–1.35 (bs, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 131.7 (CH); 129.4 (CH); 96.3 (CH_2); 67.8 (CH_2); 55.0 (CH_3); 32.6 (CH_2); 29.7 (CH_2); 29.6 (CH_2); 29.5 ($2CH_2$); 29.4 (CH_2); 29.1 (CH_2); 26.2 (CH_2); 24.6 (quint., J = 19.1 Hz, CD_2); 12.9 (hept., J = 19.1 Hz, CD_3) ppm. IR (film) υ: 2927, 2850, 2218, 1467, 1038, 916 cm^{-1}. MS (EI) *m/z* (%): 261 [(M)$^{+}$, 2]; 229 (51); 228 (53); 149 (29); 142 (46); 135 (57); 130 (68); 115 (77); 95 (72); 74 (70); 45 (100); 41 (72).

[13,13,14,14,14-$^{2}H_5$]-(*E*)-11-Tetradecen-1-ol (6)

A mixture of 0.63 g (2.39 mmol) of **5** in 5 ml of 10% HCl in methanol was stirred overnight at rt. The solvent was stripped off and the resulting crude was treated with water, extracted with hexane, dried over $MgSO_4$ and concentrated. After purification on column chromatography over silica gel eluting with hexane:ether 4:1, stereochemically pure **6** was obtained

(0.49 g, 95%) as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.39 (m, 2H); 3.62 (t, J = 6.6 Hz, 2H); 1.95 (m, 2H); 1.55 (m, 2H); 1.26–1.40 (bs, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 131.7 (CH); 129.3 (CH); 62.8 (CH_2); 32.7 (CH_2); 32.5 (CH_2); 29.6 ($2CH_2$); 29.5 ($2CH_2$); 29.4 (CH_2); 29.1 (CH_2); 25.7 (CH_2); 24.6 (quint., J = 19.1 Hz, CD_2); 12.9 (hept., J = 19.1 Hz, CD_3) ppm. IR (film) υ: 3366, 2940, 2850, 2221, 1465, 1058, 909 cm^{-1}. MS (EI) *m/z* (%): 217 [$(M)^+$, 1]; 199 (51); 171 (30); 143 (45); 114 (64); 109 (75); 100 (74); 96 (89); 82 (100); 81 (93); 72 (90); 55 (95); 41(92).

[13,13,14,14,14-$^2H_5$]-(*E*)-11-Tetradecenoic acid (7)[2]

To a solution of alcohol **6** (0.24 g, 1.10 mmol) in acetone (5 ml) were added at 0°C, 0.4 ml (1.21 mmol) of Jones reagent[8] (prepared from 3.19 g of CrO_3, 2.6 ml of H_2SO_4 conc., and 9 ml of H_2O). After stirring for 4 h at 0°C, the reaction was quenched by addition of isopropanol. The solvent was evaporated, the residue was diluted with 3N HCl and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the residue purified by column chromatography on silica gel eluting with CH_2Cl_2:MeOH 98:2 to furnish 0.21 g (84%) of acid **7** as a waxy white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.40 (m, 2H); 2.34 (t, J = 7.5 Hz, 2H); 1.96 (m, 2H); 1.63 (m, 2H); 1.27–1.36 (bs, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.5 (C); 131.8 (CH); 129.4 (CH); 34.1 (CH_2); 32.6 (CH_2); 29.6 (CH_2); 29.4 ($2CH_2$); 29.2 (CH_2); 29.1 (CH_2); 29.0 (CH_2); 24.6 (CH_2); 24.6 (quint., J = 18.7 Hz, CD_2); 12.9 (hept., J = 19.1 Hz, CD_3) ppm. IR (film) υ: 3021, 2927, 2855, 1709, 909 cm^{-1}. MS (EI) *m/z* (%): 231 [$(M)^+$, 18]; 213 (57); 171 (51); 123 (68); 110 (86); 97 (97); 73 (100); 60 (96); 43 (88); 41 (88). Elem. Anal. Calcd for $C_{14}H_{21}D_5O_2$: C, 72.67; H, 11.33. Found: C, 72.71; H, 11.45.

[13,13,14,14,14-$^2H_5$]-11-Tetradecyn-1-ol (8)

Following the same procedure described for alcohol **6** and starting from 2.5 g (9.64 mmol) of **4** and 10 ml of 10% HCl in MeOH were obtained 2.01 g (97%) of alcohol **8**, after purification on column chromatography as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.67 (t, J = 6.6 Hz, 2H); 2.17 (t, J = 6.9 Hz, 2H); 1.46–1.65 (m, 4H); 1.32–1.40 (bs, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 81.4 (C); 79.5 (C); 62.8 (CH_2); 32.6 (CH_2); 29.5 (CH_2); 29.4 (CH_2); 29.3 (CH_2); 29.0 (CH_2); 28.8 (CH_2); 25.7 (CH_2); 18.6 (CH_2); 13.2 (hept., J = 19.1 Hz, CD_3); 11.5 (quint., J = 20.0 Hz, CD_2) ppm. IR (film) υ: 3376, 2924, 2852, 2233, 1462, 1050 cm^{-1}. MS (EI) *m/z* (%): 135 (21); 128 (48); 121 (56); 114 (83); 100 (83); 95 (78); 87 (96); 79 (81); 72 (100); 67 (88); 55 (84); 41 (86). Elem. Anal. Calcd for $C_{14}H_{21}D_5O$: C, 78.01; H, 12.19. Found: C, 77.83; H, 12.40.

[13,13,14,14,14-$^2H_5$]-(*Z*)-11-Tetradecen-1-ol (9)

P-2 Nickel was prepared as previously described[4]. Under hydrogen atmosphere, alcohol **8** (0.9 g, 4.18 mmol) was added to a 10% catalyst solution in ethanol (5 ml). After stirring for 1.5 h at room temperature, the solution was filtered through Celite and the filtrate repeatedly washed with hexane. The solvents were evaporated and the crude diluted again with hexane. The organic layer was washed with brine, dried over $MgSO_4$ and the solvent removed to afford 0.89 g (98%) of stereochemically pure **9** as colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.33 (m, 2H); 3.63 (t, J = 6.6 Hz, 2H); 2.00 (m, 2H); 1.56 (m, 2H); 1.25–1.40 (bs, 14H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 131.3 (CH); 129.3 (CH); 62.9 (CH_2); 32.7 (CH_2); 29.7 (CH_2); 29.6 (CH_2); 29.5 ($2CH_2$); 29.4 (CH_2); 29.2 (CH_2); 27.0 (CH_2); 25.7 (CH_2); 19.5 (quint., J = 19.1 Hz, CD_2); 13.2 (hept., J = 19.1 Hz, CD_3) ppm. IR (film) υ: 3337, 2919, 2858, 1465, 1052 cm^{-1}. MS (EI) *m/z* (%): 217 [$(M)^+$, 2]; 199 (54); 171 (39); 143 (54); 123 (68); 114 (69); 109 (79); 95 (91); 87 (84); 82 (100); 67 (95); 55 (96); 41 (93).

[13,13,14,14,14-$^2H_5$]-(*Z*)-11-Tetradecenoic acid (10)[2]

The same procedure applied for oxidation of alcohol **6** was followed. Thus, starting from 0.25 g (1.15 mmol) of alcohol **9** in acetone (7 ml) and 0.5 ml of Jones reagent (1.27 mmol) were obtained 0.23 g (86%) of the expected acid **10** as a pale yellow oil, after purification on column chromatography eluting with CH_2Cl_2:MeOH (98:2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.35 (m, 2H); 2.34 (t, J = 7.5 Hz, 2H); 2.01 (m, 2H); 1.63 (m, 2H); 1.25–1.32 (bs, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.6 (C); 131.4 (CH); 129.3 (CH); 34.1 (CH_2); 29.7 (CH_2); 29.4 ($2CH_2$); 29.2 (CH_2); 29.0 (CH_2); 27.1 (CH_2); 24.6 (CH_2); 19.5 (quint., J = 19.1 Hz, CD_2); 13.3 (hept., J = 18.6 Hz, CD_3) ppm. IR (film) υ: 3025, 2929, 2856, 1709, 909 cm^{-1}. MS (EI) *m/z* (%): 231 [$(M)^+$, 12]; 213 (40); 171 (41); 129 (47); 110 (78); 97 (91); 83 (86); 73 (86); 69 (89); 55 (100); 44 (78). Elem. Anal. Calcd for $C_{14}H_{21}D_5O_2$: C, 72.67; H, 11.33. Found: C, 72.67; H, 11.48.

[13,13,14,14,14-$^2H_5$]-Tetradecan-1-ol (11)[10]

A suspension of 10% Pd/C (0.35 g, 0.39 mmol) in EtOH (25 ml) was purged with H_2. Then, compound **8** (0.84 g, 3.90 mmol) was added and the mixture stirred for 2 h at rt. The solution was filtered through Celite and the filtrate washed with hexane. The solvents were evaporated and the crude was purified by column chromatography on silica gel eluting with hexane:ether (75:25) to afford 0.78 g (91%) of labelled alcohol **11** as a white solid. M.p. = 35–36°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.64 (t, J = 6.6 Hz, 2H); 1.57 (m, 2H); 1.25–1.37 (bs, 20H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 62.9 (CH_2); 32.7 (CH_2); 29.7 (CH_2); 29.6 ($2CH_2$); 29.4 (CH_2); 25.7 (CH_2); 22.0 (quint., J = 19.1 Hz, CD_2); 13.7 (hept., J = 19.5 Hz, CD_3) ppm. IR (film) υ: 3329, 2918 cm^{-1}. MS (EI) *m/z* (%): 201 [$(M\text{-}18)^+$, 4]; 173 (22); 144 (22); 130 (44); 111 (78); 97 (90); 83 (100); 70 (94); 55 (92); 41 (87). Elem. Anal. Calcd for $C_{14}H_{25}D_5O$: C, 76.64; H, 13.78. Found: C, 76.72; H, 14.07.

[13,13,14,14,14-$^2H_5$]-Tetradecanoic acid (12)[10]

The same procedure followed for oxidation of alcohol **6** was applied. Starting from 0.26 g (1.18 mmol) of alcohol **11** in acetone (5 ml) and 0.5 ml of Jones reagent (1.30 mmol), acid **12** (0.24 g, 85%) was obtained as a white solid, after purification on column chromatography over silica gel eluting with CH_2Cl_2:MeOH (98:2). M.p. = 52–53°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.34 (t, J = 7.5 Hz, 2H); 1.63 (m, 2H); 1.25–1.33 (bs, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 180.1 (C); 29.7 (CH_2); 29.6 ($2CH_2$); 29.4 (CH_2); 29.2 (CH_2); 29.0 (CH_2); 24.7 (CH_2); 22.0 (quint., J = 19.1 Hz, CD_2); 13.7 (hept., J = 19.5 Hz, CD_3) ppm. IR (film) υ: 3022, 2918, 2850, 1707, 908 cm^{-1}. MS (EI) *m/z* (%): 233 [$(M)^+$, 3]; 190 (4); 129 (19); 73 (97); 60 (100); 55 (61); 41 (64).

Conclusions

In summary, penta-deuterated fatty acids have been synthesized in a very convenient manner in good to excellent yields and their application to pheromone glands of *S. littoralis* confirm the pheromone composition and biosynthesis of our current strain.

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