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Difluoropalmitic Acids as Potential Inhibitors of the Biosynthesis of the Sex Pheromone of the Egyptian Armyworm *Spodoptera littoralis*—IV†

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Abstract—2,2-, 3,3- and 4,4-Difluoropalmitic acids (**1–3**) have been synthesized and fully characterized. Acids **2** and **3** were prepared through fluorination of the corresponding dithioacetal-protected ketoesters followed by enzymatic saponification. The acids **1–3** were evaluated in vivo as inhibitors of the β-oxidation step of the biosynthesis of (*Z,E*)-9,11-tetradecadienyl acetate, the major component of the sex pheromone of the Egyptian armyworm *Spodoptera littoralis*. Only, the 2,2- and 3,3-derivatives, i.e. those containing the two fluorine atoms at the positions involved in the chain-shortened step, have been found to be active, the activity being similar to or lower than that displayed by the corresponding monofluorinated acids. Copyright © 1996 Elsevier Science Ltd

Introduction

Biosyntheses of insect sex pheromones in Lepidoptera are regulated by several key processes, particularly chain shortening and desaturation.[2] To our knowledge, the chain-shortening step, which implies successive loss of acetyl-CoA units, has not been studied yet in insects but it is presumably similar to that occurring in vertebrates, which involves a β-oxidation process localized in the peroxisomes.[3] In many insects this process has been found to be crucial, like in the orange tortix moth *Argyrotaenia citrana*,[4] the cabbage looper *Trichoplusia ni*[5] or the pink bollworm *Pectinophora gossypiella*.[6]

Inhibition of insect pheromone biosynthesis is a potentially useful tool in the search for new strategies in insect control. However, very few reports on this subject have been found in the literature,[7–9] although development of inhibitors of β-oxidation processes has been pursued for many years for their potential hypoglycemic activity.[10] The major component of the sex pheromone of the Egyptian armyworm *Spodoptera littoralis*, (*Z,E*)-9,11-tetradecadienyl acetate, has been postulated to be originated through chain shortening of palmitic acid into myristic acid through an acyl-CoA dehydrogenase.[11] We have previously prepared a variety of monofluorinated,[12] acetylene and cyclopropane fatty acids,[13] structurally related to palmitic acid, and their activity tested both in vitro and in vivo.[1] Some of the compounds emerged good inhibitors of this process. In the same vein, we now want to report on preparation of new difluorinated palmitic acids as potential inhibitors of the β-oxidation process by blockage of the 2, 3 and 4 positions of the parent fatty acid. The in vivo activity of the compounds is also presented.

Key words: Difluoropalmitic acids, inhibition, biosynthesis, sex pheromone, *Spodoptera litoralis*.

†Dedicated to the memory of Professor Félix Serratosa (1925–1995).

Results and Discussion

Introduction of fluorine into organic molecules may cause profound effects on the parent molecule due to the intrinsic features of the halogen.[14] These include high electronegativity, which induces deep changes to the reactivity of vicinal functional groups, small atomic volume similar to hydrogen and high C—F bond energy (107 kcal/mol), which makes particularly difficult metabolization of fluorinated products. For these reasons, incorporation of fluorine atom(s) into bioactive organic compounds has been considered for the design of irreversible enzyme inhibitors.[15]

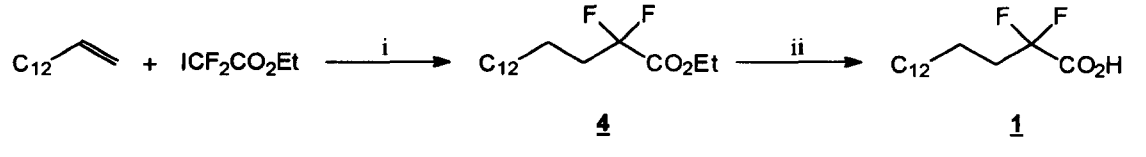
As cited above, the β-oxidation process involves the successive loss of acetyl group(s) from long-chain fatty acids as acetyl-CoA units. Consequently, inhibition of the process may occur at any stage between the initial transformation of the fatty acids into their CoA esters and the final conversion into acetyl-CoA. So far, the inhibition mechanism of very few processes has been studied in detail, i.e. inhibition of acyl-CoA synthetase, carnitine palmitoyltransferase, acyl-CoA dehydrogenase and acyl-CoA thiolase.[16] Continuing our studies of inhibition of the biosynthesis of the major component of the Egyptian armyworm *S. littoralis*, we have

synthesized 2,2-, 3,3- and 4,4-difluoropalmitic acid (**1–3**), in order to establish the effect of the presence of a second fluorine atom located at nearby positions to the ester group. Moreover, these fluorinated acids and/or esters are also important since they are versatile starting materials to prepare difluoromethylene analogues of peptides.[17a]

2,2-Difluorocarboxylic acids or esters have been prepared by a variety of methods, including photoreaction of Barton esters with 1,1-dichloro-2,2-difluoroethylene followed by hydrolysis with $AgNO_3/H_2O$–THF,[17b] reaction of iododifluoroacetate–copper system with organic halides,[18] Reformatsky–Claisen reaction of allyl chlorodifluoroacetate,[19] fluorination of α-ketoesters with SF_4 or (dialkylamino)sulfur trifluoride (DAST),[20] or copper-initiated addition of iododifluoroacetates to alkenes followed by reduction with $Zn/NiCl_2$,[21] among others. We decided to apply Yang and Burton's procedure[21] and obtained ethyl 2,2-difluorohexadecanoate (**4**) in 70% yield. The compound had already been prepared by these authors in similar yield (64%). Hydrolysis of the ester under basic conditions (LiOH/THF : H_2O) afforded difluoroacid **1** in 69% yield, with no dehydrofluorination product being detected (Scheme 1).

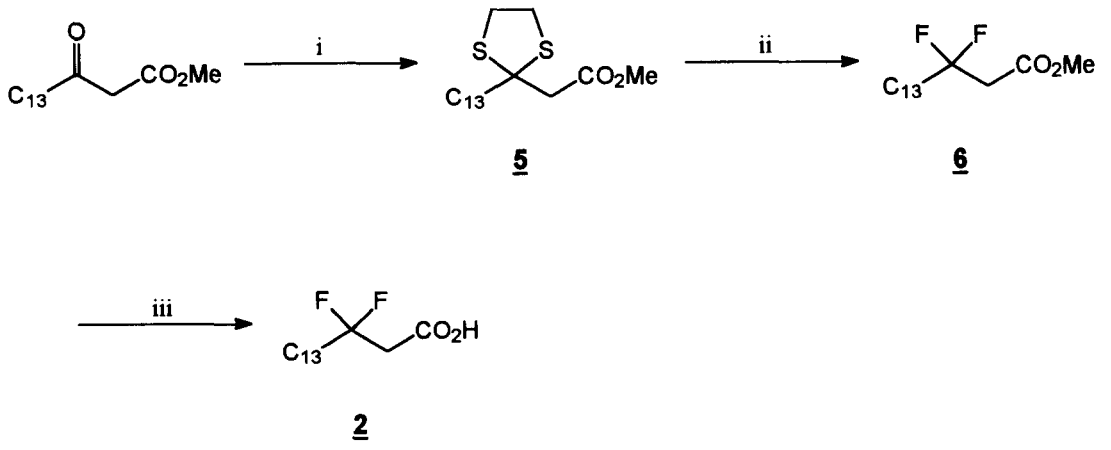
3,3-Difluoroacid (**2**) was envisaged to proceed from the corresponding methyl 3-oxohexadecanoate, obtained by acylation of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) with tetradecanoyl chloride.[12] Direct transformation of a carbonyl into a difluoromethylene group has been accomplished by using a number of fluorinating agents, like DAST,[20a] SF_4,[22] MoF_6[23] or SeF_4.[24] However, the reaction of DAST with a highly enolizable β-ketoester, such as methyl 3-oxohexadecanoate, occurs through the enol form of the ester with the final formation of methyl 2-fluoro-3-diethylsulfinamoyloxy-2-hexadecenoate.[25] Therefore, we decided to protect the carbonyl group to effect the above transformation. In this regard, several methods have been disclosed using hydrazones,[26] 1,3-dithiolanes[27] or ketoximes.[28] These procedures were, however, applied to alkyl or aryl ketones or aldehydes and in no case fluorination of a protected β-ketoester was reported. To obtain difluorinated ester **6** we decided to protect the carbonyl as the corresponding 1,3-dithiolane. Thus, reaction of methyl 3-oxohexadecanoate with 1,2-ethanedithiol in the presence of boron trifluoride-diethyl ether complex in $CHCl_3$ afforded ester **5** in excellent yield (89%). However, reaction of **5** with 1,3-dibromo-5,5-dimethylhydantoin (DBH) and pyridinium poly(hydrogen fluoride) in anh. CH_2Cl_2[27a] afforded difluoroester **6** in a modest 32% isolated yield. Obviously, the concomitant presence in the molecule of a reactive ester group limits the success of the reaction, but in any case this procedure is shorter and uses more readily accessible starting materials than the one previously reported.[29] Saponification of the ester **6** was futile under acid (*p*-TsOH/H_2O/diglyme) or basic conditions (LiOH/THF/H_2O), yielding complex mixtures, dehydrofluorinated compound or unreacted material. Reaction with formic acid in diglyme[30] or benzyl alcohol/*p*-TsOH[29] were also unsuccessful. In the same manner, treatment of **6** with lipase MY (Meito Sangyo Co.) in water or buffer soln at 37 °C for 24 h also yielded the starting material, although this process had proved successful for saponification of 3-monofluoroesters.[31] On the other hand, the alternative route, i.e. saponification of **5** (K_2CO_3/MeOH) to afford the corresponding acid (91% yield), followed by reaction of the acid with DBH/tetrabutylammonium trifluoride[32] or DBH/HF·Py, equally failed. After screening some other enzymes (*Chromobacterium viscosum*, lipase PS and lipase from *Rhyzopus arrhizus*), we could successfully achieve saponification of the ester with lipase from *R. arrhizus* at 40 °C for 24 h to obtain acid **2** in 47% yield (Scheme 2).

For the synthesis of 4,4-difluoropalmitic acid (**3**) we hypothesized that a synthetic sequence similar to that for the 3,3-isomer would have more chances to succeed for two reasons. First, because location of the fluorine atoms one carbon further from the ester group would make interaction between the halogens and the ester more difficult. Second, because position 2 is no longer simultaneously activated by the difluoromethylene and the ester groups, which would make easier the saponification of the difluorinated ester **10** on route to the desired acid **3**. Therefore, we required methyl 4-oxohexadecanoate (**8**) as the starting product. This ketoester has been cited in the literature, but not readily synthesized.[33] We decided to apply Sorokin's method, which involved the intermediate preparation of dichloroenone **7**.[34] Formation of ketone **7** from tridecanoyl chloride and propargyl chloride/$AlCl_3$ in CH_2Cl_2 took place as expected in 70% yield. We experienced problems following Sorokin's method,[34] however, in the transformation of dichloroenone **7** into ketoester **8**, since in many instances the ester was obtained contaminated with other intermediates of the reaction. Completion of the reaction was achieved simply by refluxing with 5% aq HCl to obtain **8** in satisfactory yield (57%). Following the same procedure as for **5**, protection of the carbonyl as the 1,3-dithiolane occurred as expected to afford ester **9** in 76% yield. Difluorination of **9** according to Katzenellenbogen[27a] yielded the difluorinated ester **10** in a modest 37%



4 **1**

Scheme 1. (i) Zn-$NiCl_2$·$6H_2O$/THF (70%); (ii) LiOH/THF:H_2O (69%).



Scheme 2. (i) $(CH_2SH)_2$, $BF_3{\cdot}OEt_2/CHCl_3$ (89%); (ii) DBH, HF·Py/CH_2CL_2, −78 °C (32%); (iii) lipase from *R. arrhizus*/H_2O, 40 °C (47%).

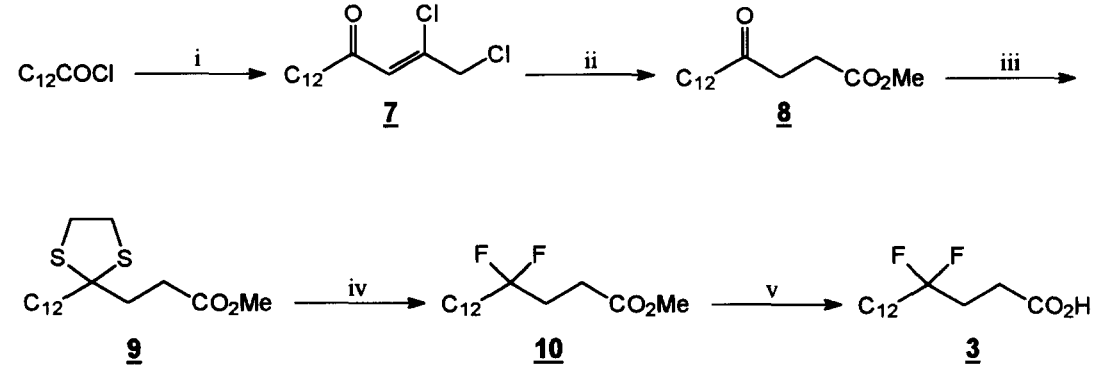
yield. This result confirms that this method is not suitable when a reactive ester group is present in the molecule, as cited above. Saponification of the ester **10** occurred in a satisfactory manner in the presence of lipase MY at 37 °C for 24 h to afford difluorinated acid **3** in 51% yield (Scheme 3).

The biological activity of compounds **1**, **2** and **3** as inhibitors of the β-oxidation of palmitic to myristic acid has been determined in in-vivo bioassays. A solution of the potential inhibitor was topically applied to the pheromone gland along with [16,16,16-$^2H_3$]-palmitic acid in DMSO. The extent of inhibition was calculated by the relative decreased intensity of the ion of *m/z* 245 of labeled methyl myristate in comparison with the control (absence of inhibitor). Five different concentrations were tested, i.e. 0.1, 0.4, 1, 2 and 5 μg/insect were assayed. The most active compound was 2,2-difluoropalmitic acid (**1**), which showed an IC_{50} of 4.4×10^{-3} μmol/insect, the same order of activity formerly displayed in vivo by 3-fluoro- and 4-fluoropalmitic acids.[1] Although the 2-fluoroderivative was not tested in vivo, it elicited in vitro very slightly lower activity than the corresponding 3-fluoroacid. This suggests that introduction of a second fluorine atom at position 2 of the molecule does not apparently affect the inhibitory potency of the monofluorinated acid. Acid **2** was, surprisingly, only significantly active at 5 μg/insect (% inhibition = 50.3 ± 7.8 SEM; $p = 0.002$, unpaired two-tailed t test), i.e. ca. fivefold less active than **1** and the corresponding 3-monofluorinated acid. In this case, therefore, introduction of a new fluorine at position 3 provokes a decrease of the inhibitory potency of the acid. The 4,4-difluoroderivative **3**, on the other hand, showed no significant activity at the maximum dose of 5 μg/insect.

In summary, difluorinated acids **1–3** have been synthesized, the latter for the first time, and their biological activity tested. Among them, only compounds **1** and **2**, containing two fluorine atoms at the positions involved in the chain-shortening step, have been found to be active as inhibitors of this process.

Experimental

Boiling points are uncorrected. Elemental analyses were determined on Carlo–Erba models 1106 and 1500. IR spectra were recorded on a Bomem MB-120 with FT spectrophotometer. ^1H and ^{13}C NMR spectra were determined in $CDCl_3$ solution on a Varian Gemini 200 or Varian Unity 300 spectrometer, operating at 200 and 300 MHz for ^1H, respectively, and



Scheme 3. (i) HC≡CCH_2Cl, $AlCl_3/CH_2Cl_2$, 10 °C (70%); (ii) (a) Na_2CO_3/MeOH, (b) 5% HCl (57%); (iii) $(CH_2SH)_2$, $BF_3{\cdot}OEt_2$/CHCl3 (76%); (iv) DBH, HF·Py/CH_2Cl_2, −78 °C (37%); (v) lipase MY/H_2O, 37 °C (51%).

at 50 and 75 MHz for ^{13}C. The values are expressed in δ scale relative to TMS. ^{19}F NMR spectra were recorded on a Varian Unity 300 instrument at 282 MHz and the values are reported relative to trichlorofluoromethane. Low-resolution MS were run on a HP 5995 mass spectrometer using a SPB-5 30 m × 0.32 μm i.d. fused silica capillary column. GLC analyses were performed on Carlo–Erba model 4130, equipped with a FID detector, using a SE-54 50 m × 0.32 μm i.d. fused silica capillary column and hydrogen as carrier gas. Reactions requiring anhydrous conditions were carried out under N_2 or Ar atmosphere. Commercial analytical-grade reagents were from Aldrich Chemie (Steinheim, Germany), Fluka Chemie AG (Buchs, Switzerland) or Fluorochem Ltd (Derbyshire, U.K.).

Ethyl 2,2-difluorohexadecanoate (4). This compound was prepared according to the method of Yang.[21] Thus, starting from $NiCl_2 \cdot 6H_2O$ (0.2 g, 0.84 mmol), Zn (0.65 g, 10 mmol), THF (10 mL), 1-tetradecene (1.96 g, 10 mmol), ethyl iododifluoroacetate (1.3 g, 5 mmol) and 1 drop of water, the expected ester **4** was obtained (1.15 g, 70%) after purification by column chromatography.

2,2-Difluorohexadecanoic acid (1). A solution of ethyl 2,2-difluorohexadecanoate (**4**) (0.2 g, 0.625 mmol), lithium hydroxide (0.053 g, 1.26 mmol) in a 2:1 mixture of THF:H_2O (5 mL) was stirred overnight at room temperature. The solvent was removed under vacuum and the residue acidified with 2% HCl, extracted with CH_2Cl_2 (3 × 10 mL), washed with H_2O (3 × 10 mL) and dried ($MgSO_4$). After removal of the solvent, column chromatography on silica gel eluting with hexane:diethyl ether (7:3) afforded 0.125 g (69%) of difluorinated acid **1**. Calcd for $C_{16}H_{30}O_2F_2$: C, 65.72; H, 10.34. Found: C, 65.97; H, 10.17. IR: ν 3450, 2920, 2848, 1751, 1463, 1186 cm^{-1}. ^1H NMR: δ 8.36 (b, 1H, COOH), 2.09 (m, 2H, CH_2CF_2), 1.50 (m, 2H, $CH_2CH_2CF_2$), 1.25 (b, 24H, $12CH_2$), 0.88 (t, J = 7 Hz, 3H, CH_3). ^{13}C NMR: δ 168.1 (t, J = 36.45 Hz, C-1), 116.29 (t, J = 247.35, C-2), 34.30 (t, J = 22.65 Hz, C-3), 31.92 (C-14), 29.7–29.07 (C-5 to C-13), 22.69 (C-15), 21.33 (C-4), 14.10 (C-16). ^{19}F NMR: δ −107.16 (t, J = 15.8 Hz).

Methyl 3-oxohexadecanoate ethylene dithioacetal (5). To a stirred solution of methyl 3-oxohexadecanoate[12] (0.21 g, 0.74 mmol) in $CHCl_3$ (2 mL) were added 1,3-propanedithiol (0.125 mL, 1.49 mmol) and $BF_3(OEt)_2$ (0.115 mL, 0.93 mmol) under Ar. The solution was stirred for 24 h, the mixture diluted with hexane (3 mL) and washed with 3 × 3 ml portions of $NaHCO_3$ satd soln, 15% NaOH soln and brine. The organic extract was dried ($MgSO_4$) and the solvent stripped off. Purification by column chromatography on silica gel (5% diethyl ether in hexane) yielded dithiolane **5** (0.24 g, 89%) as a white crystalline solid, mp < 25 °C. Calcd for $C_{19}H_{36}S_2O_2$: C, 63.28; H, 10.06; S, 17.78. Found: C, 63.39; H, 10.09; S, 17.78. IR: ν 2923, 2852, 1741, 1434 cm^{-1}. ^1H NMR: δ 3.67 (s, 3H, $COOCH_3$), 3.27 (s, 4H, SCH_2CH_2S), 3.02 (s, 2H, $CH_2CO_2CH_3$), 2.07 (m, 2H, $CH_2CSCH_2CH_2$), 1.46 (m, 2H, CH_2CH_2CS), 1.23 (b, 20H, $10CH_2$), 0.85 (t, J = 7 Hz, 3H, CH_3). ^{13}C NMR: δ 170.4 (C-1), 67.05 (C-3), 51.51 (OCH_3), 47.79 (C-2), 42.58 (C-4), 39.49 (C-1′, C-2′), 31.85 (C-14), 29.62–29.29 (C-6 to C-13), 27.10 (C-5), 22.62 (C-15), 14.05 (C-16). MS (EI): *m/z* (%) 360 (M^+, 2), 287 (12), 177 (100).

Methyl 3,3-difluorohexadecanoate (6). A similar procedure to that described for fluorination of non-functionalized dithiolanes was applied.[27a] Thus, a soln of 1,3-dibromo-5,5-dimethylhydantoin (170 mg, 0.22 mmol) in dry CH_2Cl_2 (5 mL) was stirred under Ar and cooled to −78 °C. Then, pyridinium poly(hydrogen fluoride) (200 μL) was added followed by dropwise addition of dithiolane **5** (138 mg, 0.38 mmol). The reaction mixture was stirred for 30 min, diluted with hexane and filtered through a polyethylene syringe filled with basic alumina (act. III). Subsequent column chromatography on silica gel eluting with mixtures of hexane:diethyl ether yielded 37 mg (32%) of difluoro compound **6**. IR: ν 2923, 2854, 1731, 1438, 1234 cm^{-1}. ^1H NMR: δ 3.71 (s, 3H, OCH_3), 2.89 (t, J = 15 Hz, $CH_2CO_2CH_3$), 1.97 (m, 2H, CH_2CF_2), 1.46 (m, 2H, $CH_2CH_2CF_2$), 1.23 (b, 20H, $10CH_2$), 0.86 (t, J = 7 Hz, 3H, CH_3). ^{13}C NMR: δ 167.56 (C-1), 122.24 (t, J = 241.1 Hz, C-3), 52.15 (OCH_3), 41.60 (t, J = 28.65 Hz, C-2), 36.05 (t, J = 24 Hz, C-4), 31.9 (C-14), 29.66–29.20 (C-6 to C-13), 22.68 (C-15), 22.17 (t, J = 4.3 Hz, C-5), 14.11 (C-16). ^{19}F NMR: δ −94.45 (qt, J = 16.1 Hz). MS (CI, CH_4): *m/z* (%) 347 (M^+ + 41,3), 335 (M^+ + 24, 20), 306 (M^+ + 1, 12), 287 (100), 267 (85).

3,3-Difluorohexadecanoic acid (2). A mixture of ester **6** (5.6 mg, 0.0183 mmol), lipase from *R. arrhizus* (Fluka, EC 3.1.1.3; 30 mg) and water (660 μL) was shaken in a thermostatized bath at 40 °C and 80 U/min for 24 h. The mixture was filtered and washed with CH_2Cl_2. The filtrate was acidified with 1 N HCl and extracted again with CH_2Cl_2. The organic solvents were combined and evaporated to leave a residue, which was purified by column chromatography on silica gel eluting with CH_2Cl_2:MeOH (99:1) to yield 2.5 mg (47%) of acid **2** as a white solid, mp 65–68 °C. IR: ν 3400, 2925, 2854, 1724, 1467 cm^{-1}. ^1H NMR: δ 2.94 (t, J = 14.7 Hz, CH_2CO_2H), 2.07 (m, 2H, CH_2CF_2), 1.55 (m, 2H, $CH_2CH_2CF_2$), 1.23 (b, 20H, $10CH_2$), 0.86 (t, J = 6.9 Hz, 3H, CH_3). ^{19}F NMR: δ −94.58 (qt, J = 15.5 Hz).

1,2-Dichlorohexadec-2-en-4-one (7). The procedure described by Sorokin was applied.[34] To a solution of tridecanoyl chloride (2 g, 8.6 mmol) and $AlCl_3$ (1.15 g, 8.62 mmol) in CH_2Cl_2 (13 mL) was added dropwise at −10 °C propargyl chloride (0.62 g, 8.32 mmol). The mixture was stirred for 1 h and 3 h at rt. After pouring into ice, the organic layer was separated, washed with satd aq $NaHCO_3$ and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel eluting with hexane:diethyl ether (99:1) to yield 1.88 g (70%) of dichloroenone **7**, as a mixture of isomers

(*E*/*Z*) 90/10. IR: ν 3039, 1700, 1604, 1045 cm^{-1}. ^1H NMR: δ (*E* isomer) 6.51 (s, 1H, COCH=CCl), 4.74 (s, 2H, CH_2Cl), 2.47 (t, $J = 7$ Hz, 2H, CH_2CO), 1.59 (m, 2H, $C\underline{H}_2CH_2CO$), 0.87 (t, $J = 7$ Hz, 3H, CH_3). (*Z* isomer): 6.6 (s, 1H, COCH=CCl), 4.2 (s, 2H, CH_2Cl), 2.6 (t, $J = 7$ Hz, 2H, CH_2CO). ^{13}C NMR: δ (mixture of isomers) 197.83 (C-4), 147.70, 128.15 (CH=CCl), 44.76 (C-1), 42.96 (C-5), 31.9 (C-14), 29.7–29.01 (C-7 to C-13), 23.67 (C-6), 22.66 (C-15), 14.09 (C-16).

Methyl 4-oxohexadecanoate (8). We have modified the procedure reported by Sorokin.[34] A mixture of Na_2CO_3 (0.7 g, 6.60 mmol), 1,2-dichlorohexadec-2-en-4-one (**7**) (1.35 g, 4.4 mmol) in MeOH (4.4 mL) was refluxed for 24 h. The mixture was filtered off and the precipitate washed with diethyl ether. To the organic solution was then added 5% aq HCl (7 mL) and the mixture stirred at rt for 2 h and refluxed for 1 h more. The organic phase was separated and the aqueous layer extracted with diethyl ether (3 × 10 mL). The combined organic phases were washed with brine and dried ($MgSO_4$) to leave a residue, after evaporation of the solvent, which was chromatographed on silica gel eluting with hexane:ethyl acetate (98:2) to yield 0.71 g (57%) of compound **8**. Calcd for $C_{17}H_{32}O_3$: C, 71.78; H, 11.34. Found: C, 71.80; H, 11.50. IR: ν 2923, 2852, 1741, 1718, 1465 cm^{-1}. ^1H NMR: δ 3.66 (s, 3H, OCH_3), 2.71 (m, 2H, $COCH_2$), 2.57 (m, 2H, $CH_2CO_2CH_3$), 2.43 (t, $J = 7.2$ Hz, 2H, CH_2CO), 1.57 (m, 2H, $C\underline{H}_2CH_2CO$), 1.24 (b, 18H, $9CH_2$), 0.86 (t, $J = 7$ Hz, 3H, CH_3). ^{13}C NMR: δ 209.1 (C-4), 173.3 (C-1), 51.74 (OCH_3), 42.8 (C-2), 36.98 (C-3), 31.9 (C-14), 29.58–29.18 (C-5 and C-7 to C-13), 23.79 (C-6), 22.66 (C-15), 14.09 (C-16).

Methyl 4-oxohexadecanoate ethylene dithioacetal (9). To a stirred solution of ester **8** (0.1 g, 0.35 mmol) in $CHCl_3$ (1 mL) were added 1,3-propanedithiol (59 μL, 0.7 mmol) and $BF_3(OEt)_2$ (52 μL, 0.42 mmol). The mixture was stirred for 18 h and worked up as described for **5** to yield dithiolane **9** (97 mg, 76%). Calcd for $C_{19}H_{36}S_2O_2$: C, 63.28; H, 10.06; S, 17.78. Found: C, 63.22; H, 10.04; S, 17.60. IR: ν 2923, 2852, 1739, 1463, 1437, 1168 cm^{-1}. ^1H NMR: δ 3.64 (s, 3H, OCH_3), 3.23 (s, 4H, SCH_2CH_2), 2.57 (m, 2H, $C\underline{H}_2CO_2CH_3$), 2.17 (m, 2H, $CC\underline{H}_2SCH_2CH_2$), 1.87 (m, 2H, $C\underline{H}_2CSCH_2CH_2$), 1.50 (m, 2H, $C\underline{H}_2CH_2CS$), 1.22 (b, 18H, $9CH_2$), 0.85 (t, $J = 7$ Hz, CH_3). ^{13}C NMR: δ 173.83 (C-1), 70.84 (C-4), 51.61 (OCH_3), 44.53 (C-2), 39.87 (C-1′, C-2′), 37.47 (C-3), 31.88 (C-14), 31.44 (C-5), 29.72–29.31 (C-6 to C-13), 22.65 (C-15), 14.08 (C-16). MS (CI, NH_3): *m/z* (%) 378 ($M^+ + 18$, 15), 361 ($M^+ + 1$, 100).

Methyl 4,4-difluorohexadecanoate (10). Starting from dithiolane **9** and applying the same procedure as described for **6**, difluorinated ester **10** was obtained (25 mg, 37%). IR: ν 2952, 2925, 2854, 1745, 1438, 1197, 934, 734 cm^{-1}. ^1H NMR: δ 3.67 (s, 3H, OCH_3), 2.51 (m, 2H, $CH_2CO_2CH_3$), 2.15 (m, 2H, CF_2CH_2), 1.80 (q, $J = 17$ Hz, 2H, CH_2CF_2), 1.44 (m, 2H, $C\underline{H}_2CH_2CF_2$), 1.24 (b, 18H, $9CH_2$), 0.86 (t, $J = 7$ Hz, 3H, CH_3). ^{13}C NMR: δ 173 (C-1), 124.2 (t, $J = 240$ Hz, C-4), 51.8 (OCH_3), 36.7 (t, $J = 25$ Hz, C-3), 31.9 (C-14), 31.5 (C-5), 26.8, 22.2 (t, $J = 5$ Hz, C-2 and C-6), 29.7–29.3 (C-7 to C-13), 22.7 (C-15), 14.1 (C-16). ^{19}F NMR: δ −100.44 (qt, $J = 16.6$ Hz). MS (CI, CH_4): *m/z* (%) 347 ($M^+ + 41$, 3), 335 ($M^+ + 29$, 10), 287 ($M^+ - 19$, 100). MS (EI): *m/z* (%) 291 (2), 287 (19), 286 (31), 275 (9), 235 (2), 233 (5).

4,4-Difluorohexadecanoic acid (3). A mixture of methyl 4,4-difluorohexadecanoate (12 mg, 0.04 mmol), lipase MY (Meito) (28 mg) and H_2O (0.6 mL) was capped and shaken in a thermostatized bath at 37 °C and 80 U/min. The progress of the reaction was monitored by TLC. When the conversion was completed (24 h), the mixture was filtered off and the enzyme washed with diethyl ether. The aqueous phase was acidified with 1 N HCl and extracted with diethyl ether (3 × 5 mL). The organic phase was washed with brine and dried ($MgSO_4$). After removal of the solvent, the crude was purified by column chromatography on silica gel, eluting with hexane:ethyl acetate (98:2) to yield 4,4-difluorohexadecanoic acid (**3**) (6 mg, 51%). Calcd for $C_{16}H_{30}O_2F_2$: C, 65.72; H, 10.34. Found: C, 65.75; H, 10.22. IR: ν 3044, 2920, 2842, 1694, 1467, 1183, 911, 732 cm^{-1}. ^1H NMR: δ 2.57 (m, 2H, $C\underline{H}_2CO_2CH_3$), 2.17, 2.15 (dq, $J = 17$ Hz, 2H, CF_2CH_2), 1.81 (q, $J = 17$ Hz, 2H, CH_2CF_2), 1.44 (m, 2H, $C\underline{H}CH_2CF_2$), 1.23 (b, 18H, $9CH_2$), 0.86 (t, $J = 7$ Hz, 3H, CH_3). ^{13}C NMR: δ 177.4 (C-1), 124.2 (t, $J = 239.5$ Hz, C-4), 36.74 (t, $J = 24.8$ Hz, C-3), 31.91 (C-14), 31.28 (t, $J = 25.6$ Hz, C-5), 26.76, 22.26 (t, $J = 5$ Hz, C-2 and C-6), 29.7–29.3 (C-7 to C-13), 22.68 (C-15), 14.1 (C-16). ^{19}F NMR: δ −100.54 (qt, $J = 16.6$ Hz).

In-vivo bioassays

Application of labeled substrates. For each experiment a total of 63 insects in three batches were used, two for every concentration of the inhibitor and one for control. A solution of the potential inhibitor at different concentrations ranging from 3.4×10^{-10} to 1.7×10^{-8} mol/gland in 0.1 μL of DMSO was topically applied to the pheromone gland 1 h before the onset of the scotophase. Control insects were treated only with DMSO. After 30 min of absorption, 1 μg of [16,16,16-$^2H_3$]palmitic acid, dissolved in 0.1 μL of DMSO, was applied to the glands and the insects released and kept in the dark for 2 h.

Fatty acid methyl esters analyses. The pheromone glands were dissected and treated in groups of 3 with 400 μL of 0.5 N KOH in methanol for 1 h at room temperature and quenched with 400 μL of 1 N HCl. To this mixture was added 10 ng of methyl tridecanoate in 10 μL of hexane per gland as internal standard and the organic material extracted with 1 mL of hexane. The labeled fatty acid methyl esters solutions were frozen at

−20 °C for a maximum of 3 days and concentrated to 5–10 μL for GC–MS analysis.

GC–MS analyses. Analyses were carried out in a Fisons MD 800 GC–MS spectrometer operating at 70 eV in the splitless mode and under SIM (single ion monitoring) acquisition conditions. The purge valve was opened 0.8 min after injection, the carrier gas utilized was helium and the operating pressure was 14 psi. A HP-5 25 m × 0.20 mm i.d. crosslinked 5% phenylmethyl silicone fused silica capillary column was used. The column was initially set at 80 °C for 1 min and then programmed at 5 °C/min until 185 °C, kept at this temperature for 5 min, programmed at 3 °C/min until 200 °C and at 10 °C/min until a final temperature of 300 °C, which was maintained for 10 min. The following ions were selected: 228 (methyl tridecanoate, as standard), 245 (d_3-14:Me), 273 (d_3-16:Me) and 242, and 270 for the parent non-deuterated compounds.

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