Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 7, pp 1267–1274, 1997

PII: S0968-0896(97)00072-2
0968-0896/97 $17.00 + 0.00

Identification and Biosynthesis of (*E,E*)-10,12-Tetradecadienyl Acetate in *Spodoptera littoralis* Female Sex Pheromone Gland

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Abstract—A minor component of the sex pheromone gland of the Egyptian cotton leafworm, *Spodoptera littoralis*, has been identified as (*E,E*)-10,12-tetradecadienyl acetate. Structural elucidation has been carried out by isobutane–chemical ionization mass spectrometry of the fatty acyl biosynthetic precursor-derived methyl ester. To assign the stereochemistry of the double bonds, the four isomers of both 10,12-tetradecadienyl acetates and methyl 10,12-tetradecadienoates have been synthesized and their gas chromatography retention times and mass spectra have been compared to those of the corresponding natural compounds. Mass-labeling experiments showed that the (*E,E*)-10,12-tetradecadienoyl moiety is biosynthetically derived from (*Z*)-11-tetradecenoic acid in the insect pheromone gland. © 1997 Elsevier Science Ltd.

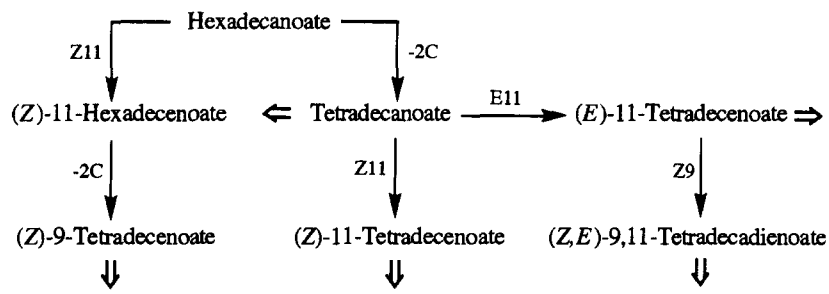
Introduction

The biosynthesis of the female sex pheromone of the Egyptian cotton leafworm moth, *Spodoptera littoralis*, was unveiled in our laboratories[1] by application of mass-labeled tracers and GC-MS analysis of both pheromone and biosynthetic intermediate extracts. As shown in Scheme 1, the main component, (*Z,E*)-9,11-tetradecadienyl acetate, and other C14 acetates, such as (*Z*)-9-tetradecenyl, (*E*)-11-tetradecenyl, (*Z*)-11-tetradecenyl and tetradecyl acetates,[2] are originated from a common precursor, hexadecanoic acid, by β-oxidation, selective desaturation, reduction, and acetylation reactions. The occurrence of a minor component of *S. littoralis* pheromone blend had also been reported by Dunkelblum et al.[2] These authors tentatively assigned the (*Z,Z*)-9,11-tetradecadienyl acetate structure to this compound. This minor acetate was also present in the pheromone gland of the *S. littoralis* strain reared in our laboratories.

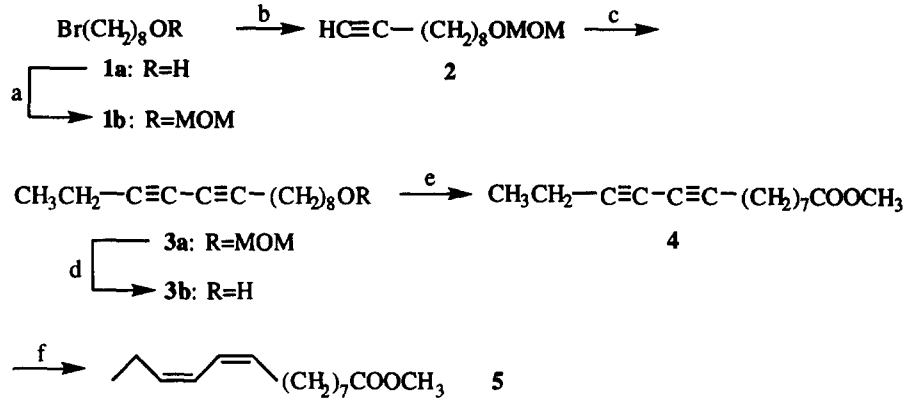
In this article we report on the assignment of the (*E,E*)-10,12-tetradecadienyl acetate structure to this component, and the study of its biosynthetic formation.

Results and Discussion

In apparent agreement with the suggestion of Dunkelblum et al.[2] GC-MS analysis of this minor component in pheromone gland extracts showed the occurrence of ions at *m/z* 252 ($M^{\cdot+}$), 192 ($M^{\cdot+}-60$) and a base peak at *m/z* 67 (data not shown), which is in accord with a 9,11-tetradecadienyl acetate. To compare both the GC retention time and MS fragmentation pattern of the natural compound with an authentic standard, (*Z,Z*)-9,11-tetradecadienyl acetate was synthesized following the sequence of reactions depicted in Scheme 2. Protection of 8-bromo-1-octanol (**1a**) as the methoxymethyl ether[3] followed by reaction with lithium acetilyde[4] gave rise to terminal acetylene **2**, which was transformed into the diyne **3b** by Cadiot–Chodkiewick reaction[5] with 1-bromo-1-butyne and further hydrolysis of the protective group. Oxidation of diynol **3b** with pyridinium dichromate in DMF[6] and esterification of the resulting acid furnished ester **4**, which was stereoselectively reduced into the (*Z,Z*)-conjugated diene **5** with Zn(Cu/Ag) following standard procedures.[7] A small aliquot part was reduced with $LiAlH_4$ and further acetylated with acetyl chloride to give the corresponding acetate.



Scheme 1. Biosynthetic pathway of *S. littoralis* sex pheromone. The reactions involved are: *Z*11-desaturation (*Z*11), *Z*9-desaturation (*Z*9), *E*11-desaturation (*E*11), and β-oxidation (-2C). Open arrows indicate reduction and acetylation.



Scheme 2. Reagents: (a) LiBr/*p*-TsOH/dimethoxymethane (91%); (b) lithium acetilyde/DMSO (90%); (c) CuI/Pr-NH_2/NH_2OH-HCl, **1b**/MeOH, then 1-bromo-1-butyne/Et_2O (33%); (d) 10% HCl/MeOH (66%); (e) pyridinium dichromate/DMF, then K_2CO_3/CH_3I/DMF (44%); (f) Zn(Cu/Ag)/MeOH/H_2O (30%).

Although the mass spectrum of synthetic (*Z,Z*)-9,11-tetradecadienyl acetate exhibited the same fragmentation pattern (Scheme 2) as the natural compound (data not shown), its GC retention time on a nonpolar column was clearly different from that of the natural material. This discrepancy led us to the application of Einhorn et al.'s[8] procedure for the direct determination of double-bond positions in long-chain conjugated dienes by CI-MS using isobutane as reagent gas. The mass spectra of straight-chain conjugated dienes display, besides the ions corresponding to $M^{\cdot+}$, (MH^+) and ($M^{\cdot+}$ + C_4H_9), diagnostically useful ions A^+ and B^+ (Fig. 1) at *m/z* values depending on the position of the conjugated diene system along the chain. Unfortunately, however, these ions are not intense in the case of diene acetates.[8] Since methanolysis of pheromone gland lipids gives rise to methyl fatty esters derived from the acyl pheromone precursors,[9] we prepared a methanolyzed extract and performed the isobutane-CI-MS analysis of the resulting natural methyl tetradecadienoate. As shown in Figure 1, the most abundant B^+ fragment for the unknown dienoate corresponded to the peak at *m/z* 213, which suggested the occurrence of double bonds at C10 and C12. Therefore, on the basis of these analyses, the unknown compound appeared to be a methyl-10,12-tetradecadienoate and, consequently, the pheromone extract component a 10,12-tetradecadienyl acetate.

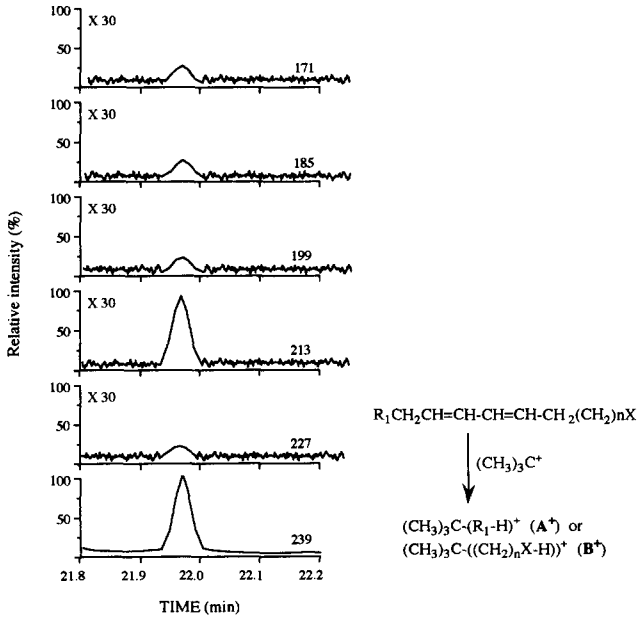
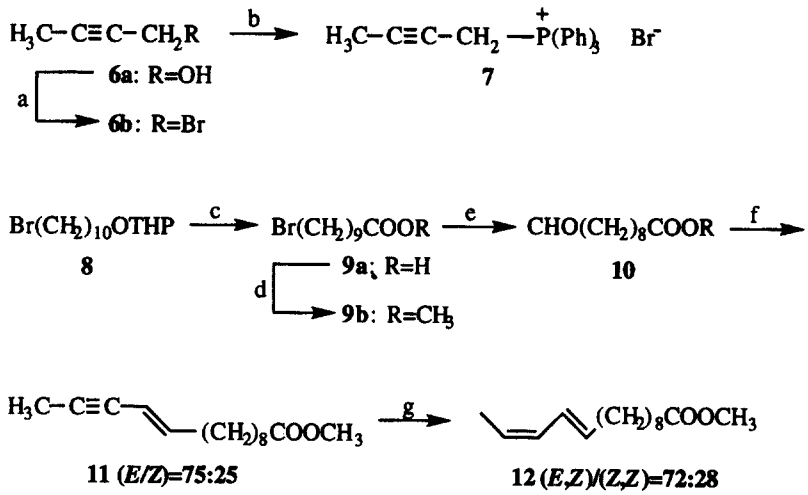
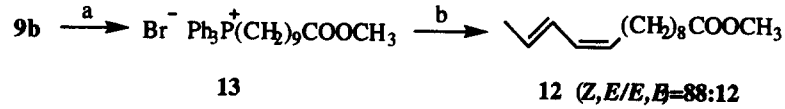


Figure 1. Selected ion-monitoring traces for double-bond location in conjugated methyl tetradecadienoate (X=COOMe) by chemical ionization MS using isobutane as reagent gas. The MH^+ ion is that at *m/z* 239. Ions B^+ correspond to possible double bonds at positions C11–C13 (227), C10–C12 (213), C9–C11 (199), C8–C10 (185), and C7–C9 (171).

To further confirm this assignment and to establish the stereochemistry of the double bonds at C10 and C12, suitable synthetic standards were prepared. Preparation of (*E,Z*)- and (*Z,Z*)-isomers was carried out as shown in Scheme 3. Phosphonium salt **7**[10] was prepared from 1-bromo-2-butyne (**6b**), which was in turn obtained from 2-butyn-1-ol (**6a**) following a reported procedure.[4] On the other hand, aldehyde **10** was prepared by oxidation of bromo ester **9b** with pyridine *N*-oxide[11] and compound **9b** was in turn synthesized from **8** by Jones oxidation and further methylation. Wittig reaction of aldehyde **10** with the phosphorane obtained by treatment of **7** with potassium *t*-butoxide gave rise to **11** as a mixture of isomers (*E*/*Z*) (75:25) (^{1}H NMR and capillary GC). The predominance of the (*E*)-isomer was expected, since a stabilized ylide is formed from phosphonium salt **7**.[12] The stereochemistry of the major enynoate was established on the basis of the coupling constant between C10-H and C11-H in the signals corresponding to C10-H in both isomers,[13] which is higher ($J_{10,11}$ = 15.8 Hz) in the (*E*)-isomer (major) than in the (*Z*)-isomer (minor, $J_{10,11}$ = 10.6 Hz). Stereoselective reduction of (*E*/*Z*)-**7** using Zn-Cu(Ag) according to reported procedures[7] afforded methyl 10,12-tetradecadienoate as a mixture of isomers (*E,Z*):(*Z,Z*) (72:28) (capillary GC). Finally, to obtain the corresponding acetates, a small sample was reduced with



Scheme 3. Reagents: (a) Ref 4 (86%); (b) Ph_3P (82%); (c) CrO_3/H_2SO_4/acetone (99%); (d) 10% H_2SO_4/MeOH (77%); (e) pyridine N-oxide/$NaHCO_3$/toluene (65%); (f) **7**/K *t*-BuO/THF (61%); (g) Zn(Cu/Ag)/MeOH/H_2O (28%).



Scheme 4. Reagents: (a) Ph_3P/DMF (83%); (b) K *t*-BuO/THF, then (*E*)-2-butenal/THF (44%).

lithium aluminum hydride and acetylated with acetyl chloride.

The synthesis of the corresponding (*Z,E*)- and (*E,E*)-isomers ((*Z,E*)- and (*E,E*)-**12**) was similarly accomplished, as shown in Scheme 4. Wittig reaction of (*E*)-2-butenal with the nonstabilized ylide derived from treatment of salt **13** with potassium *t*-butoxide furnished methyl 10,12-tetradecadienoate (**12**), with an isomeric ratio (*Z,E*):(*E,E*) (80:20). Again, formation of the (*Z,E*) isomer as predominant was expected on the basis of mechanistic considerations[12] and confirmed by ^1H NMR, in which the pattern observed for the vinylic protons was similar to that of the vinylic protons of (*Z,E*)-11,13-hexadecadienyl acetate, which was obtained through a stereoselective reaction.[13] An analytical sample was reduced and acetylated as indicated above to obtain the corresponding acetates.

In order to elucidate the stereochemistry of the double bonds, a mixture of the two pairs of isomers synthesized as described above was prepared and coinjected with a pheromone gland extract on two columns of different polarities. As shown in Figure 2, the natural acetate coeluted with (*E,E*)-10,12-tetradecadienyl acetate from both columns. Additionally, a mixture of the four isomers of the methyl ester precursors was also prepared from the two pairs of isomers obtained and coinjected with a methanolyzed gland extract on the same two columns. Again, the natural unknown dienoate coeluted with methyl (*E,E*)-10,12-tetradecadienoate (data not shown). Future electrophysiological and behavioral studies in our laboratories will show whether this compound is perceived by the males and it is part of the pheromone communication system.

As mentioned in the Introduction, the biosynthetic pathway of *S. littoralis* pheromone blend occurs, as established by Martinez et al.,[1] as shown in Scheme 1. In the mass-labeling experiments reported in this previous article, we found incorporation of mass label in the then unidentified (*E,E*)-10,12-tetradecadienyl acetate and its diene acyl precursor from deuterated hexadecanoic and tetradecanoic acids, but not from deuterated (*Z*)-11-

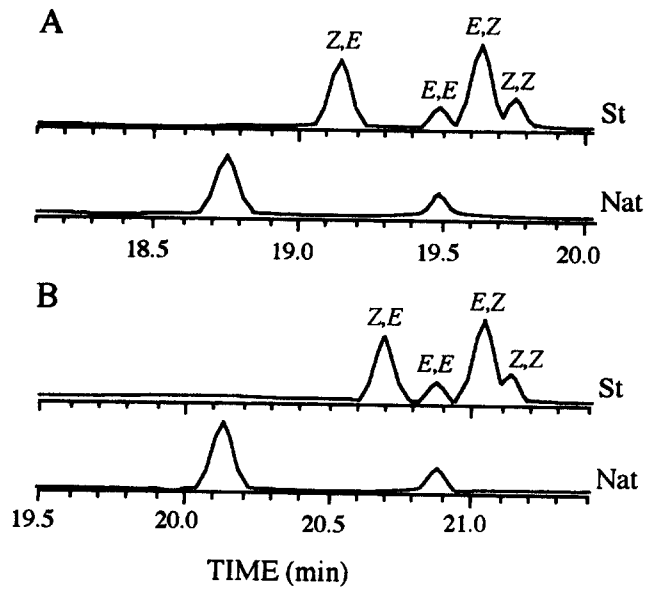
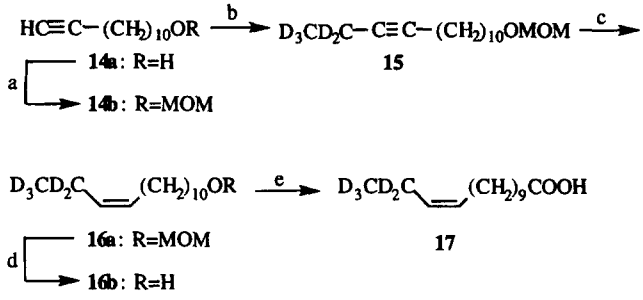


Figure 2. Capillary GC traces corresponding to a mixture of the four isomers of synthetic 10,12-tetradecadienyl acetate (St) and to a natural sex pheromone extract (Nat) using A, an HP-1 nonpolar column and B, a BP-20 polar column. In the pheromone extract analyses, the compound eluting first corresponds to (*Z,E*)-9,11-tetradecadienyl acetate.



Scheme 5. Reagents: (a) LiBr/*p*-TsOH/dimethoxymethane (98%); (b) CH_3Li/THF, then ICD_2CD_3/HMPA (99%); (c) H_2/Pd-$BaSO_4$/quinoline (96%); (d) 10% HCl/MeOH (83%); (e) CrO_3/H_2SO_4/acetone (67%).

hexadecenoic or (*E*)-11-tetradecenoic acids. These previous results suggested that (*Z*)-11-tetradecenoic acid might be the precursor of the (*E,E*)-10,12-tetradecadienoyl system. In this article we confirm this hypothesis in mass-labeling experiments using (13,13,14,14,14-$^2H_5$) (*Z*)-11-tetradecenoic acid (**17**) as tracer.

Deuterated (*Z*)-11-tetradecenoic acid **17** was prepared as outlined in Scheme 5. Alkylation of terminal acetylide of **14b** with perdeuterated ethyl iodide under standard conditions[14] afforded acetylene **15**, which was partially hydrogenated[15] to furnish **16a**. Deprotection and final Jones oxidation gave the expected labeled acid **17**.

As shown in Figure 3, methanolyzed sex pheromone lipidic extracts from insects treated with **17** contained labeled methyl (*E,E*)-10,12-tetradecadienoate, as indicated by the occurrence in the GC-MS traces of the ($M^{\cdot+}$ + 4) ion at *m/z* 242, which eluted 0.08 min before the natural compound. This ion was not observed in control extracts prepared from pheromone glands that were only treated with dimethylsulfoxide. This result demonstrates that the (*E,E*)-10,12-tetradecadienoyl system is biosynthetically originated from (*Z*)-11-tetradecenoic acid. Similar transformations occur in the biosynthesis of *Bombyx mori*,[16] *Manduca sexta*,[17] and *Cydia pomonella*[18] sex pheromones. In the first two cases, (*Z*)-11-hexadecenoic acid gives rise to 10,12-hexadecadienoyl moieties, whereas in *Cydia pomonella*, (*E*)-9-dodecenoic acid is transformed into (*E,E*)-8,10-dodecadienoic acid. The exact mechanism of this kind of conversion is still unknown, although it might

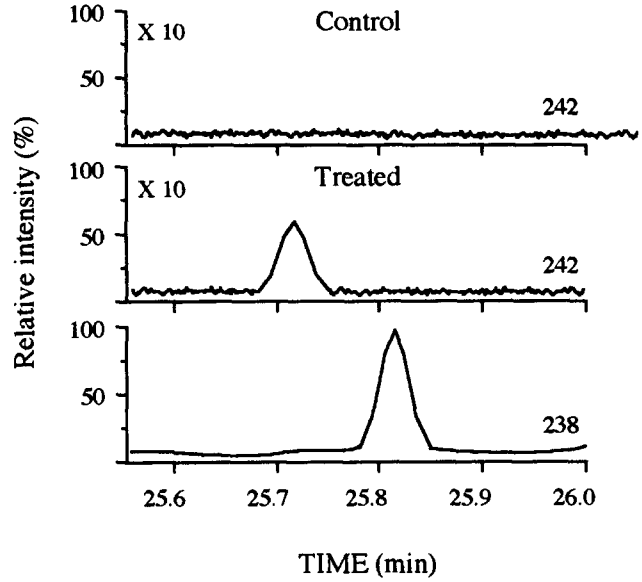
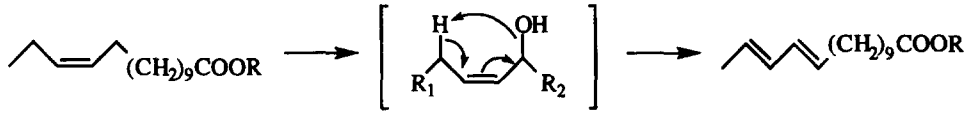


Figure 3. Incorporation of a deuterium label from topically applied (13,13,14,14,14-$^2H_5$)-(*Z*)-11-tetradecenoic acid into methyl-(*E,E*)-10,12-tetradecadienoate in pheromone glands of *S. littoralis* females. GC-MS analyses were performed on both treated and control extracts by selecting ions at *m/z* 238 and 242, corresponding to $M^{\cdot+}$ for the natural methyl-(*E,E*)-tetradecadienoate and to $M^{\cdot+}$ for the corresponding methyl ester with four deuterium atoms.

involve, as suggested by Lofstedt and Bengtsson,[18] allylic oxidation of the monoenoic intermediate followed by 1,4-elimination of water (Scheme 6). Further work following this approach is in progress at our laboratory.

In summary, in contrast to a previous report,[2] we have unambiguously identified the structure of a minor compound present in the sex pheromone gland of *S. littoralis* females as (*E,E*)-10,12-tetradecadienyl acetate and have proved that (*Z*)-11-tetradecenoic acid is its biosynthetic precursor. The result of this biosynthetic study adds more complexity to the already sophisticated system of desaturases involved in the biochemical pathway of *S. littoralis* female sex pheromone. Thus, whereas (*E*)-11-tetradecenoic acid is (*Z*)-9 desaturated to give the (*Z,E*)-9,11-tetradecadienoyl system,[1] (*Z*)-11-tetradecenoic acid is converted, through a still unknown mechanism, to the (*E,E*)-10,12-tetradecadienoyl moiety. It is becoming apparent that as our knowledge of the biosynthesis of lepidopteran sex pheromones increases, the more interesting these biological models become when studying desaturase reactions of fatty acids.



$R_1=CH_3$ and $R_2=(CH_2)_8COOR$ or

$R_1=(CH_2)_8COOR$ and $R_2=CH_3$

Scheme 6. Proposed mechanism for the transformation of (*Z*)-11-tetradecenoic acid into (*E,E*)-10,12-tetradecadienoic acid in *S. littoralis* pheromone glands.

Experimental

THF was distilled from Na/benzophenone under N_2. Reactions sensitive to oxygen and moisture were conducted under Ar atmosphere. Purification of products by column chromatography was performed on Merck silica gel. TLC was carried out on precoated silica gel Merck 60 F_{254} (0.25 mm) sheets. Elemental analyses were obtained with a Carlo Erba model 1106. FT-IR spectra were recorded in film on a Michelson Bomem MB-120 spectrometer. ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ with either a Varian XL200 or Varian Unity 300 spectrometers at 200 or 300 MHz, respectively, for ^1H and 50 or 75 MHz for ^{13}C, respectively.

Mass-labeling experiments were performed as previously reported.[1] Briefly, two-day-old virgin *S. littoralis* females were immobilized and their pheromone glands extruded and treated with a solution of tracer **17** in dimethylsulfoxide (0.1 μL, 10 mg/mL). Pheromone glands were dissected 2 h after the applications. For pheromone analysis, glands were extracted with hexane (20 μL/gland) at room temperature for 2 h. For analysis of intermediates, the tissues were soaked with chloroform:methanol (2:1) at 4 °C for 14 h and the lipidic extract thus obtained was base methanolyzed as described elsewhere[1] to obtain the pheromone precursors as fatty acid methyl esters. Both pheromone and intermediate extracts were analyzed by GC-MS under selected ion monitoring (SIM) conditions using a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass-selective detector. The system was equipped with a nonpolar Hewlett-Packard HP-1 capillary column (30 m × 0.20 mm ID), or a polar SGE BP-20 (30 m × 0.20 mm ID) using the following temperature programs: from 120 to 180 °C at 5 °C/min, and then to 260 °C at 2 °C/min after an initial delay of 2 min (nonpolar column); or from 100 to 230 °C at 5 °C/min, and then to 260 °C at 10 °C/min after an initial delay of 2 min (polar column).

Chemical ionization mass spectrometry was performed with a Finnigan MAT Incos XL spectrometer with the following conditions: source temperature = 100 °C, filament current = 200 μA and isobutane pressure = 0.3 torr. The mass spectrometer was coupled to a Varian Star 3400 gas chromatograph equipped with a HP-1 capillary column, which was programmed from 80 to 220 °C at 5 °C/min, and then to 300 °C at 10 °C/min.

Synthesis of standard methyl esters and acetates

11,13-Dioxa-1-tetradecyne (2). 8-Bromo-1-octanol (**1a**) (8 g, 38 mmol), LiBr (0.66 g, 7.6 mmol) and *p*-TsOH (0.65 g, 3.4 mmol) were dissolved in dimethoxymethane (0.76 mL, 1.16 mol) and the mixture was stirred at room temperature for 16 h. After this time, a satd solution of NaCl was added and the mixture was extracted with hexane. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated to yield 8.8 g (35 mmol, 91%) of compound **1b**. Compound **2** was prepared in 90% yield from **1b** (6 g, 240 mmol), and lithium acetilyde in DMSO, following literature procedures.[4] IR 2931, 2858, 1463, 1145, 1110, 1049 cm^{-1}. ^1H NMR δ 1.21 (b, 8H, C5-H to C8-H), 1.38 (c, 2H, C4-H), 1.45 (c, 2H, C9-H), 1.82 (t, J = 2.5 Hz, 1H, C1-H), 2.04 (dt, J = 6.9 and 2.4 Hz, 2H, C3-H), 3.22 (s, 3H, C14-H), 3.38 (t, J = 6.6 Hz, 2H, C10-H), 4.45 (s, 2H, C12-H); ^{13}C NMR δ 18.10 (C3), 25.93 (C9), 28.22, 28.43, 28.82, 29.05, 29.48 (C4 to C8), 54.67 (C14), 67.45 (C10), 67.98 (C1), 84.22 (C2), 96.04 (C12).

15,17-Dioxa-3,5-octadecadiyne (3a). A solution of 0.1 g (0.5 mmol) of CuI and 0.05 g (0.75 mmol) of $NH_2OH{\cdot}HCl$ in 1 mL (9.6 mmol) of $PrNH_2$, cooled at 0 °C, was treated with 1 g (5 mmol) of **2** dissolved in 8 mL of degassed MeOH. The mixture was stirred at 0 °C for 2 h and then was added a solution of 1-bromo-1-butyne (1.2 g, 9 mmol) in 2 mL of Et_2O. The reaction mixture was stirred at 55 °C for 24 h, cooled to room temperature and treated with 1 N HCl and extracted with hexane. Evaporation of the solvent gave an oil, which was treated with a 2% solution of $AgNO_3$ in EtOH (10 mL) to remove the unreacted starting material. The resulting suspension was filtered, solvent was evaporated from the filtrate, and the residue was diluted with hexane and washed with brine. Solvent removal gave 0.39 g (1.5 mmol, 33%) of pure diacetylene **3a**. IR 2929, 2854, 2358, 1461, 1143, 1110, 1049 cm^{-1}. ^1H NMR δ 1.04 (t, J = 7.4 Hz, 3H, C1-H), 1.22 (b, 8H, C9-H to C12-H), 1.42 (c, 4H, C8-H, and C13-H), 2.13 (c, 4H, C2-H, and C7-H), 3.24 (s, 3H, C18-H), 3.40 (t, J = 6.4 Hz, C14-H), 4.45 (s, 2H, C16-H); ^{13}C NMR δ 12.61 (C2), 13.18 (C1), 18.88 (C7), 25.92 (C13), 28.08, 28.52, 28.80, 29.03, 29.48 (C8 to C12), 54.71 (C18), 65.09 (C4), 65.15 (C5), 67.48 (C14), 77.12 (C6), 78.15 (C3), 96.07 (C16).

9,11-Tetradecadiyn-1-ol (3b). A solution of **3a** (0.39 g, 1.5 mmol) in 10% HCl in MeOH (10 mL) was stirred at room temperature until complete disappearance of the starting material by TLC. Extraction with CH_2Cl_2 afforded a crude that was purified by flash chromatography (hexane:Et_2O, 3:2) to furnish 0.21 g (1 mmol, 66%) of diynol **3b**. IR 3350, 2931, 2856, 1458, 1429, 1315, 1056 cm^{-1}. ^1H NMR δ 1.07 (t, J = 7.2 Hz, 3H, C14-H), 1.24 (b, 8H, C3-H to C6-H), 1.46 (c, 4H, C2-H, and C7-H), 2.18 (c, 4H, C8-H, and C13-H), 3.52 (t, J = 6.4 Hz, 2H, C1-H); ^{13}C NMR δ 12.60 (C13), 13.13 (C14), 18.87 (C8), 25.47 (C3), 28.06, 28.50, 28.82, 29.03 (C4 to C7), 32.44 (C2), 62.53 (C1), 64.56 (C11), 65.09 (C10), 77.32 (C9), 78.36 (C12),

Methyl-9,11-tetradecadiynoate (4). Compound **3b** (60 mg, 0.3 mmol) was treated with a solution of 0.92 g (2.4 mmol) of pyridinium dichromate in DMF (3 mL). After stirring for 16 h at room temperature, the mixture was acidified with 3 N HCl and extracted with CH_2Cl_2. The organic layer was washed with water and then with brine, and dried (Na_2SO_4). Solvent removal afforded a crude that was dissolved in 1 mL of DMF and treated with K_2CO_3 (0.16 g, 1.16 mmol) for 10 min and then with CH_3I (0.072 mL, 1.15 mmol). The reaction mixture was

stirred at room temperature for 16 h. After this time, extraction with hexane furnished ester **4** (30 mg, 0.13 mmol, 44%). IR 2935, 2856, 2238, 1739, 1434, 1261, 1170, 800 cm^{-1}. ^1H NMR δ 1.13 (t, J = 7.2 Hz, 3H, C14-H), 1.29 (c, 6H, C4-H to C6-H), 1.48 (c, 2H, C7-H), 1.60 (c, 2H, C3-H), 2.22 (c, 4H, C8-H, and C13-H), 2.28 (t, J = 7.5 Hz, 2H, C2-H), 3.65 (s, 3H, OCH_3); ^{13}C NMR δ 12.88 (C13), 13.39 (C14), 19.12 (C8), 24.85 (C3), 28.20, 28.58, 28.69, 28.93 (C4 to C7), 34.02 (C2), 51.43 (OCH_3), 64.61 (C11), 65.21 (C10), 77.49 (C9), 78.63 (C12), 174.23 (C-1).

Methyl-(*Z,Z*)-9,11-tetradecadienoate (5). Activated Zn (0.7 g, 10.7 mmol) was suspended in water (4 mL) and was added, under Ar, 70 mg (0.35 mmol) of $Cu(OAc)_2 \cdot H_2O$. After 15 min of stirring, $AgNO_3$ (70 mg, 0.4 mmol) was added and stirring was maintained for a further 30 min. After this time, the metal was filtered under vacuum and Ar, and washed, subsequently, with water (2 × 9 mL), MeOH (2 × 9 mL), acetone (2 × 9 mL), and Et_2O (2 × 9 mL). The Zn thus prepared was immediately transferred to a flask and suspended in 4 mL of MeOH:H_2O (1:1), and a solution of **4** (30 mg, 0.13 mmol) in MeOH (1 mL) was added. The mixture was stirred at room temperature under Ar. After total disappearance of the starting diyne by GC, the mixture was filtered through celite using MeOH. The solvent was removed under vacuum, the residue was treated with water and extracted with hexane, and washed with brine. Removal of the solvent gave 9 mg (0.037 mmol, 30%) of **5**. IR: 2927, 2854, 1741, 14,360, 1431, 1259, 1197, 1172, 910, 734 cm^{-1}. ^1H NMR δ 0.98 (t, J = 7.5 Hz, 3H, C14-H), 1.60 (c, 2H, C3-H), 2.16 (c, 4H, C8-H, and C13-H), 2.29 (t, J = 7.3 Hz, 2H, C2-H), 3.65 (s, 3H, OCH_3), 1.25–1.40 (c, 8H, C4-H to C7-H), 5.31–5.49 (c, 2H, C10-H, and C13-H), 6.15–6.28 (c, 2H, C11-H, and C12-H); ^{13}C NMR δ 14.19 (C14), 20.77 (C13), 24.92 (C3), 27.40 (C8), 29.04, 29.07, 29.09, 29.52 (C4 to C7), 34.08 (C2), 51.44 (OCH_3), 122.94 (C11), 123.48 (C10), 132.00 (C9), 133.67 (C12), 174.31 (C1).

2-Butynyltriphenylphosphonium bromide (7). 1-Bromo-2-butyne (**6b**) (2.21 g, 16.6 mmol, 86%) was prepared as described[4] from 2-butynol (**6a**) (1.35g, 19.3 mmol). Stirring of compound **6b** and Ph_3P (4.36 g, 16.6 mmol) at room temperature for 16 h gave a suspension that was filtered and thoroughly washed with hexane to obtain 5.4 g (13.7 mmol, 82%) of salt **7** as a light-yellow solid (mp 92–93 °C). IR: 2939, 2449, 1438, 1239, 1112 cm^{-1}. ^1H NMR: δ 1.64 (dt, J = 6.4 and 2.7 Hz, 3H, C4-H), 5.00 (dq, J = 14.8 and 2.7 Hz, 2H, C1-H), 7.62–7.91 (c, 15H, ArH); ^{13}C NMR: δ 3.49 (d, J = 3.2 Hz, C4), 17.98 (d, J = 56.0 Hz, C1), 65.98 (d, J = 13.7 Hz, C2), 84.64 (d, J = 9.6 Hz, C3), 117.36 (d, J = 87.1 Hz, C1(H), 130.13 (d, J = 12.8 Hz, C2(H), 133.65 (d, J = 10.6 Hz, C3′H), 135.12 (d, J = 3.1 Hz, C4′H).

10-Bromodecanoic acid (9a). A solution of 8.6 g (86 mmol) of CrO_3 in 14.5 mL of water was cooled to 0 °C and 7.4 mL (127 mmol) of H_2SO_4 was slowly added. The reagent thus prepared was then added, at −5 °C, to a solution of **8** (5 g, 15.6 mmol) in 86 mL of acetone. After stirring overnight, acetone was evaporated and acid **9a** (3.8 g, 15.4 mmol, 99%) isolated with CH_2Cl_2. IR: 3550-2500, 2929, 2854, 1708, 1411, 1282 cm^{-1}. ^1H NMR: δ 1.25 (b, 10H, C4-H to C8-H), 1.57 (b, 2H, C3-H), 1.79 (c, 2H, C9-H), 2.29 (t, J = 7.0 Hz, 2H, C2-H), 3.34 (t, J = 6.6 Hz, 2H, C10-H); ^{13}C NMR: δ 24.34 (C3), 27.85, 28.40, 28.70, 28.84, 28.93 (C4 to C8), 32.50 (C9), 33.71 (C10), 33.87 (C2), 180.63 (C1).

Methyl-10-bromodecanoate (9b). A solution of **9a** (3.8 g, 15.4 mmol) in 10 mL of 10% H_2SO_4 in 95% MeOH was stirred under reflux for 18 h. After this time, the solvent was evaporated and the residue was extracted with hexane, washed with $NaHCO_3$ and brine, and dried over $MgSO_4$. Removal of the solvent gave an oil that was purified by flash chromatography using hexane: Et_2O (4:1) as eluent affording 3.15 g (11.9 mmol, 77%) of ester **9b**. IR: 2929, 2854, 1739, 1461, 1434, 1251, 1197, 1172 cm^{-1}. ^1H NMR: δ 1.27 (b, 10H, C4-H to C8-H), 1.59 (c, 2H, C3-H), 1.79 (quint, J = 7.6 Hz, 2H, C9-H), 2.24 (t, J = 7.6 Hz, 2H, C2-H), 3.37 (t, J = 6.8 Hz, 2H, C10-H), 3.63 (s, 3H, OCH_3); ^{13}C NMR: δ 24.43 (C3), 27.67, 28.37, 28.71, 28.70, 28.79 (C4 to C8), 32.37 (C9), 33.17 (C10), 33.46 (C2), 50.74 (OCH_3); 173.25 (C1).

Methyl-9-formylnonanoate (10). In a three-neck round-bottomed flask was placed a solution of **9b** (1.3 g, 4.9 mmol) in 7 mL of toluene, 0.94 g (9.9 mmol) of pyridine *N*-oxide and 0.83 g (9.9 mmol) of anhydrous $NaHCO_3$. The mixture was heated at 140 °C for 4 h, cooled to room temperature and the product isolated with hexane to give a brown oil. Purification by flash chromatography eluting with hexane:Et_2O (3:2) furnished 0.63 g (3.2 mmol, 65%) of pure aldehyde **10**. IR: 2929, 2855, 2719, 1737, 1436, 1172, 1102 cm^{-1}. ^1H NMR: δ 1.28 (b, 8H, C4-H to C7-H), 1.58–1.64 (c, 4H, C3-H, and C8-H), 2.28 (t, J = 5.2 Hz, 2H, C2-H), 2.40 (dt, J = 5.0 and 1.2 Hz, 2H, C9-H), 3.64 (s, 3H, OCH_3), 9.74 (t, J = 1.2 Hz, CHO); ^{13}C NMR: δ 21.39 (C8), 24.23 (C3), 28.24, 28.38, 28.43, 28.50 (C4 to C7), 32.23 (C2), 43.08 (C9), 50.47 (OCH_3), 173.09 (C1), 201.42 (CHO).

9-Methoxycarbonylnonyltriphenylphosphonium bromide (13). A solution of **9b** (1.0 g, 3.4 mmol) and triphenylphosphine (0.99 g, 3.4 mmol) in 10 mL of DMF was heated under reflux for 14 h. After cooling to room temperature CH_2Cl_2 was added and the organic layer was washed extensively with a 1 N solution of HCl, and dried. Evaporation of solvent gave an oil that was dried at 120 °C/0.1 torr to yield 1.52 g (2.83 mmol, 83%) of salt **13**. IR: 2927, 2858, 1729, 1438, 1112 750, 723, 692 cm^{-1}. ^1H NMR: δ 0.87 (b, 8H, C4-H to C7-H), 1.23 (c, 6H, C3-H, C8-H, and C9-H), 1.91 (t, J = 7.6 Hz, 2H, C2-H), 3.27 (s, 3H, CH_3O), 3.33 (c, 2H, C10-H), 7.39–7.55 (c, 15H, ArH); ^{13}C NMR: δ 21.29 (d, J = 32.7 Hz, C1), 21.75 (d, J = 23.0 Hz, C2), 23.83 (C8), 27.95, 28.04 (C4 to C7), 29.40 (d, J = 15.3 Hz, C3), 33.01 (C9), 50.45 (CH_3O), 117.28 (d, J = 86.9 Hz, C1(H), 129.61 (d, J = 12.6 Hz, C2(H), 132.62 (d, J = 10.4 Hz, C3(H), 134.16 (d, J = 3.0 Hz, C4(H), 173.20 (C10).

Methyl-(*E*)-10-tetradecen-12-ynoate (11). To a suspension of dry 2-butynyltriphenyl-phosphonium bromide (**7**) (0.74 g, 1.87 mmol) in anhydrous THF (14 mL) under argon was added potassium *t*-butoxide (0.21 g, 1.87 mmol). The mixture was stirred at room temperature for 30 min and the aldehyde **10** (0.3 g, 1.5 mmol) was added. Stirring was continued for 4 h and the reaction mixture was quenched by adding a small volume of water and extracted with hexane. The combined organic layers were washed with brine and dried to afford, after solvent removal, 0.73 g of a thick oil that was purified by flash chromatography eluting with hexane:Et_2O (3:2). Enynes **11** (0.210 g, 0.93 mmol, 61%) were thus obtained in an *E*:*Z* ratio of (4:1) (capillary GC). IR: 3018, 2991, 2925, 2854, 1739, 1465, 1434, 1195, 1172 cm^{-1}. ^1H NMR: δ 1.21 (b, 10H, C4-H to C8-H), 1.53 (c, 2H, C3-H), 1.83 (d, J = 2.0 Hz, 3H, C14-H (*E*) isomer), 1.89 (d, J = 2.0 Hz, 3H, C14-H (*Z*) isomer), 1.98 (q, J = 6.8 Hz, 2H, C9-H), 22.22 (t, J = 7.4 Hz, 2H, C2-H), 3.58 (s, 3H, OCH_3), 5.29–5.47 (c, 1H, C11-H), 5.71 (dt, J = 10.6 and 7.4 Hz, C10-H (*Z*) isomer), 5.95 (dt, J = 15.8 and 7.0 Hz, C10-H (*E*) isomer); ^{13}C NMR: δ 3.98 (C14 (*E*) isomer), 4.20 (C14 (*Z*) isomer), 24.78 (C3), 28.71, 28.89, 29.00, 29.08, 29.12 (C4 to C8 and C9 (*Z*) isomer), 32.81 (C9 (*E*) isomer), 51.30 (OCH_3), 78.31 (C12 (*E*) isomer), 83.86 (C13 (*E*) isomer), 109.17 (C11 (*Z*) isomer), 109.74 (C11 (*E*) isomer), 142.42 (C10 (*Z*) isomer), 143.17 (C10 (*E*) isomer), 174.09 (C1).

Methyl-(*E,Z*)-10,12-tetradecadienoate (*E,Z*-12). The same procedure described for the preparation of **5** was applied to diynoate **8** (0.1 g, 0.42 mmol) to obtain 40 mg of an oil. Purification by flash chromatography using hexane furnished 29 mg (0.12 mmol, 28%) of a mixture of dienes (*E,Z*):(*Z,Z*) in a ratio of 72:28 (capillary GC analysis). IR: 3018, 2925, 2854, 1741, 1652, 1434, 1195, 1170 cm^{-1}. IR: 3018, 2925, 2854, 1741, 1453, 1434, 1195, 1170 cm^{-1}. ^1H NMR: δ 1.22 (b, 10H, C4-H to C8-H), 1.59 (c, 2H, C3-H), 1.72 (c, 3H, C14-H), 2.07 (c, 2H, C9-H), 2.28 (t, J = 6.8 Hz, 2H, C2-H), 3.60 (s, 3H, OCH_3), 5.39 (c, C13-H (*E,Z*)-isomer, C10-H (*Z,Z*)-isomer and C13-H (*Z,Z*)-isomer), 5.64 (dt, J = 15.0 and 6.8 Hz, C10-H (*E,Z*)-isomer), 5.95 (tq, J = 10.8 and 1.6 Hz, C12-H (*E,Z*)-isomer), 6.24 (c, C11-H and C12-H (*Z,Z*)-isomer), 6.31 (ddq, J = 15.0, 10.8, and 1.0 Hz, C11-H (*E,Z*)-isomer); ^{13}C NMR: δ 13.10 (C14 (*Z,Z*)-isomer), 13.25 (C14 (*E,Z*)-isomer), 24.92 (C3), 27.42 (C9 (*Z,Z*)-isomer), 29.11, 29.17, 29.28, 29.37, 29.59 (C4 to C8), 32.87 (C9 (*E,Z*)-isomer), 34.08 (C2), 51.43 (CH_3O), 123.26 (C12 (*Z,Z*)-isomer), 123.86 (C11 (*E,Z*)-isomer), 124.53 (C11 (*Z,Z*)-isomer), 125.30 (C12 (*E,Z*)-isomer), 125.90 (C13 (*Z,Z*)-isomer), 129.50 (C13 (*E,Z*)-isomer), 131.92 (C10 (*Z,Z*)-isomer), 134.51 (C10 (*E,Z*)-isomer), 174.32 (C1).

Methyl-(*Z,E*)-10,12-tetradecadienoate (*Z,E*-12). The same procedure described for the preparation of **11** was applied using 9-methoxycarbonylnonyltriphenyl-phosphonium bromide (**13**) (0.65 g, 1.20 mmol) and crotonaldehyde (0.15 mL, 1.80 mmol). Purification of the reaction crude by silicagel column chromatography eluting with hexane:diethyl ether (3:2) afforded 0.139 g (44% yield) of a mixture of methyl and *t*-butyl (*Z,E*)-10,12-tetradecadienoate 1:1 (capillary GC). Isomeric ratio (*Z,E*)/(*E,E*) was (88:12) (capillary GC). IR: 3016, 2927, 2854, 1731, 1456, 1436, 1365, 1153 cm^{-1}. ^1H NMR: δ 1.26 (b, 10H, C4-H to C8-H), 1.41 (s, 9H, $C(CH_3)_3$), 1.56 (c, 2H, C3-H), 1.74 (d, J = 6.9 Hz, 3H, C14-H), 2.14 (c, 2H, C9-H), 2.17 (t, J = 7.5 Hz, 2H, C2-H *t*-Bu ester), 2.27 (t, J = 7.5 Hz, 2H, C2-H Me ester), 3.63 (s, 3H, OCH_3), 5.27 (c, C13-H), 5.68 (dt, J = 14.6 and 7.0 Hz, C10-H), 5.92 (t, J = 10.8, C12-H), 6.32 (c, C11-H); ^{13}C NMR ((*E,Z*)-isomer): δ 18.22 (C14), 24.90 (C3 Me ester), 25.06 (C3 *t*-Bu ester), 27.60 (C9), 28.07, ($C(\underline{C}H_3)_3$), 29.03, 29.15, 29.20, 29.29, 29.67 (C4 to C8), 34. 04 (C2 Me ester), 35.56 (C2 *t*-Bu ester), 51.35 (OCH_3), 79.79 (($CH_3)_3\underline{C}O$), 127.01 (C13), 128.44 and 128.87 (C11 and C12), 129.75 (C10), 173.23 (C1 *t*-Bu ester), 174.22 (C1 Me ester).

Conversion of carboxylic esters 5 or 12 into the corresponding acetates. In a vial capped with a septum was placed a suspension of $LiAlH_4$ (5–10 mg) in dry Et_2O (0.25 mL). A solution of methyl esters **5** or **12** (10–50 μg, 0.04–0.2 μmol) was added under argon at 0 °C and the reaction mixture was allowed to stand at room temperature for 1 h. After this time, the mixture was cooled to −76 °C and 1 N HCl (0.2 mL) was added. The mixture was slowly warmed to room temperature and extracted with hexane (3 × 0.2 mL). The combined organic layers were washed with water and the solvent was evaporated under a stream of nitrogen. The resulting residue was treated with acetyl chloride (50 μL) for 1 h at room temperature. The excess of reagent was then evaporated under nitrogen and the residue was dissolved in hexane (0.1 mg/mL) and used in the analyses.

Synthesis of tracer 17

13,15-Dioxa-1-hexadecyne (14b). Preparation of compound **14b** was carried out in 98% yield from 11-dodecyn-1-ol (2.0 g, 10.9 mmol) as described for compound **1b**. IR: 3303, 2927, 2854, 2113, 1465, 1145, 1110, 1043 cm^{-1}. ^1H NMR: δ 1.27 (b, 12H, C5-H to C10-H), 1.46–1.62 (c, 4H, C4-H and C11-H), 1.92 (t, J = 2.7 Hz, 1H, C1-H), 2.16 (dt, J = 6.9 and 2.7 Hz, 2H, C3-H), 3.45 (s, 3H, C16-H), 3.50 (t, J = 6.6 Hz, 2H, C12-H), 4.60 (s, 2H, C14-H); ^{13}C NMR: δ 18.38 (C3), 26.19 (C10); 28.46, 28.72, 29.06, 29.40, 29.51, 29.72 (C4 to C9), 55.07 (C16), 67.85 (C12), 68.02 (C1), 84.78 (C2), 96.36 (C14).

(1,1,2,2,2-$^2H_5$) 15,17-Dioxa-3-octadecyne (15). A 0.5 g (2.2 mmol) quantity of **14b** in 5 mL of dry THF was placed in a three-neck round-bottom flask, under argon. The resulting solution was cooled to −10 °C, and 2 mL (2.6 mmol) of a 1.3 M solution of MeLi in Et_2O was added. The mixture was stirred for 1 h at 0 °C and then 0.2 mL (2.5 mmol) of ($^2H_5$) ethyl iodide in 2.3 mL of HMPA was added at this temperature. Stirring was continued for 2 h at room temperature and the mixture was poured onto ice and extracted with hexane. The

organic layers were washed with 1 N HCl and brine, dried, and the solvent evaporated to afford 0.56 g (2.16 mmol, 99%) of **15**. IR: 2927, 2854, 2229, 2138, 2973, 1741, 1465, 1110, 1045 cm^{-1}. ^{1}HNMR: δ 1.28 (b, 12H, C7 to C12), 1.57 (c, 4H, C6 and C13), 2.11 (t, J = 6.8 Hz, 2H, C5-H), 3.45 (s, 2H, C18-H), 3.51 (t, J = 6.4 Hz, 2H, C14-H), 4.60 (s, 2H, C16-H); ^{13}C NMR: δ 11.45 (quint, J = 20.5 Hz, C2), 13.20 (hept, J = 19.1 Hz, C1), 18.59 (C5), 26.10 (C12), 28.72, 29.03, 29.31, 29.35, 29.43, 29.62 (C6 to C11), 54.87 (C18), 67.69 (C14), 79.31 (C4), 81.43 (C3), 96.21 (C16).

(1,1,2,2,2-$^2H_5$) (Z)-15,17-Dioxa-3-octadecene (16a). A suspension of 0.36 g (1.4 mmol) of **15**, 40 mg (0.37 mmol) of 10% Pd-$BaSO_4$, and 0.040 mL of quinoline in 4 mL of hexane:methanol (20:1) was placed in a hydrogenation flask. The system was purged and filled with H_2 and the reaction was allowed to proceed until the theoretical amount of H_2 had been consumed. The reaction mixture was filtered through celite, solvent was removed from the filtrate and 1 N HCl was added to the resulting residue. Extraction with hexane gave 0.35 g (1.34 mmol, 96%) of olefin **16a**. IR: 2925, 2854, 2360, 2221, 2069, 1465, 1110, 1045 cm^{-1}. ^{1}H NMR: δ 1.26 (b, 12H, C7-H to C12-H), 1.57 (c, 4H, C6-H and C13-H), 1.99 (c, 2H, C5-H), 3.47 (s, 3H, OCH_3), 3.50 (t, J = 6.6 Hz, C14-H), 4.61 (s, 2H, C16-H), 5.38 (c, 2H, C3-H and C4-H); ^{13}C NMR: δ 13.49 (hept, J = 18.6 Hz, C1), 19.49 (quint, J = 19.6 Hz, C2), 26.15 (C12), 27.02 (C5), 29.38, 29.46, 29.51, 29.53, 29.69, 29.71 (C6 to C11), 54.93 (C18), 67.77 (C14), 96.27 (C16), 129.24 (C4), 131.28 (C3).

(13,13,14,14,14-$^2H_5$) (Z)-11-Tetradecen-1-ol (16b). A solution of **16a** (55 mg, 0.2 mmol) in 10% HCl in methanol (4 mL) was stirred at room temperature for 18 h. After this time, solvent was evaporated and the residue extracted with CH_2Cl_2 to obtain 38 mg (0.18 mmol, 83%) of alcohol **16b**. A small sample was purified by column chromatography on silica gel eluting with hexane:ethyl acetate (85:15) for characterization. Anal. calcd for $C_{14}H_{23}D_5O$: C, 77.42; H, 10.60; found: C, 77.52; H, 10.64. IR: 3339, 2923, 2854, 1465, 1056 cm^{-1}. ^{1}H NMR: δ 1.25 (b, 14H, C3-H to C9-H), 1.55 (c, 2H, C2-H), 1.98 (c, 2H, C10-H), 3.65 (c, 3H, C1-H and OH), 5.31 (c, 2H, C11-H and C12-H); ^{13}C NMR: δ 13.24 (hept, J = 19.3 Hz, C14), 19.50 (quint, J = 19.1 Hz, C13), 25.74 (C3), 27.02 (C10), 29.22, 29.39, 29.47, 29.50, 29.55, 29.71 (C4 to C9), 32.92 (C2), 63.01 (C1), 129.27 (C11), 131.32 (C12).

(13,13,14,14,14-$^2H_5$) (Z)-11-Tetradecenoic acid (17). Jones oxidation of **16b** (38 mg, 0.18 mmol), following the procedure described for the preparation of **9a** afforded acid **17** (25 mg, 0.12 mmol, 67%), which was purified by column chromatography on silica gel eluting with CH_2Cl_2/methanol (97:3). Anal. calcd for $C_{14}H_{21}D_5O_2$: C, 72.73; H, 9.09; found: C, 72.76; H, 9.19. IR: 2500–3500, 2925, 2854, 2221, 2138, 2975, 1710, 1463 cm^{-1}. ^{1}H NMR: δ 1.27 (b, 12H, C4-H to C9-H), 1.60 (c, 2H, C3-H), 1.99 (c, 2H, C10-H), 2.33 (t, J = 7.5 Hz, C2-H), 5.3 (c, 2H, C11-H and C12-H); ^{13}C NMR: δ 13.29 (hept, J = 18.8 Hz, C14), 19.55 (quint, J = 19.4 Hz, C13), 24.66 (C3), 27.07 (C10), 29.04, 29.22, 29.38, 29.44, 29.74 (C4 to C9), 34.05 (C2), 129.32 (C11), 131.41 (C12), 179.96 (C1).

Acknowledgements

We thank Comisión Asesora de Investigación Científica y Técnica (Grant AGF-95-0185), Comissionat per a Universitats i Recerca from the Generalitat de Catalunya (Grant GRQ 93-8016) and SEDQ SA (Grants CDTI 94-0329 and DGTI 94-583) for financial support. The award of a predoctoral fellowship (to I.N.) by SEDQ SA is also gratefully acknowledged.

References

1. Martinez, T.; Fabriàs, G.; Camps, F. *J. Biol. Chem.* **1990**, *265*, 1381.

2. Dunkelblum, E.; Kehat, M.; Gothilf, S.; Greenberg, S.; Sklarsz, B. *Phytoparasitica* **1982**, *10*, 21, and references therein.

3. Gras, J. L.; Chang, Y. Y. K. W.; Guerin, A. *Synthesis* **1985**, 74.

4. Brandsma, L.; Verkruijsse, H. D. In *Synthesis of Acetylenes, Allenes and Cumulenes. A Laboratory Manual*; Elsevier Scientific: New York, 1981.

5. Ohba, S.; Engbersen, J. F. J. *Tetrahedron* **1991**, *47*, 9947.

6. Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.

7. Avignon-Tropis, M.; Pougny, J. R. *Tetrahedron Lett.* **1989**, *30*, 4951.

8. Einhorn, J.; Virelizier, H.; Gemal, A. L.; Tabet, J. C. *Tetrahedron Lett.* **1985**, *26*, 1445.

9. Roelofs, W. L.; Bjostad, L. *Bioorg. Chem.* **1984**, *12*, 279.

10. Khrimyan, A. P.; Makaryan, G. M.; Ovanisyan, A. L.; Wimmer, Z.; Romañuk, M.; Streinz, L.; Badanyan, S. O. *Khim. Prir. Soedin.* **1991**, 117.

11. Stowell, J. C. *J. Org. Chem.* **1970**, *34*, 244.

12. Schlosser, M. *Topics Stereochem.* **1970**, *5*, 1.

13. Camps, F.; Fabrias, G.; Gasol, V.; Guerrero, A.; Hernandez, R.; Montoya, R. *J. Chem. Ecol.* **1988**, *14*, 1331.

14. Schwarz, M.; Waters, R. M. *Synthesis* **1972**, *6*, 567.

15. Cram, D. J.; Allinger, N. L. *J. Am. Chem. Soc.* **1956**, *78*, 2518.

16. Yamaoka, R.; Taniguchi, Y.; Hayashita, K. *Experientia* **1984**, *4*, 81.

17. Fang, N. B.; Teal, P.; Doolittle, R. E.; Tumlinson, J. H. *Insect Biochem. Molec. Biol.* **1995**, *25*, 39.

18. Lofstedt, C.; Bengtsson, M. *J. Chem. Ecol.* **1988**, *14*, 903.

(*Received in U.S.A.* 19 *September* 1996; *accepted* 5 *February* 1997)