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Location of double bonds in diene and triene acetates by partial reduction followed by methylthiolation

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Abstract

Two random reduction procedures (NH_2NH_2/H_2O_2 and NH_2NH_2/O_2) were compared and conditions optimized for the reduction of two synthetic pheromone compounds (9*Z*,11*E*)-9,11-tetradecadienyl acetate and (9*Z*,12*E*)-9,12-tetradecadienyl acetate on a 300 μg scale at 60 °C. The relative amounts of the four products (completely reduced acetate, unreacted diene acetate and two monoene acetates), characterized by gas chromatography (GC) from the reaction mixture, depended on the reaction conditions. The reduction was straightforward without any detectable undesired side products. The reaction yields were reproducible with both the reducing reagents. The optimized reduction conditions thus established were utilized to reduce seven synthetic compounds (four diene and three triene acetates) on a micro scale (5 μg). In all cases, expected compounds were identified by GC–MS. After reduction, two methods were used to locate the position of double bonds in the partially reduced compounds. In the first method, the products from the above seven compounds were isolated by extraction with hexane and reacted with dimethyl disulfide to give the DMDS adducts. In the second method ("one-pot"), the reduced compounds were not isolated but instead, the solvents were evaporated and the DMDS derivatives formed. In both cases, determination of the position of the double bonds was possible by GC–MS analyses. The complete procedure (reduction and DMDS derivative formation) could be carried out on a 100 ng scale. Although neither of the partial reduction methods offered significant advantages over the other, partial reduction with NH_2NH_2/H_2O_2 was more convenient and hence should be the method of choice, together with DMDS derivative formation to locate double bonds in pheromones. In addition, a new procedure is described using ND_2ND_2/H_2O_2 and DMDS derivative formation capable of distinguishing between the double bond positions in (*Z*)-9-tetradecenyl acetate and (9*Z*,12*E*)-9,12-tetradecadienyl acetate (1:1 mixture).

Keywords: Double bond; Unsaturation; Diene acetates; Triene acetates; Partial reduction; Methylthiolation; DMDS derivatives; GC–MS; Pheromones

1. Introduction

Location of double bonds in long-chain unsaturated organic compounds is a classical problem, particularly challenging when the compounds are available only in nanogram quantities and as complex mixtures. Pheromone chemists are often confronted with this problem. The methods available for double bond location have been reported [1–6]. One of the best methods available entails the addition of dimethyl disulfide (DMDS) to the double bond followed by electron-impact mass spectrometry of adducts. This method has been used extensively for locating double bonds in alkenes [7,8], acetates [9], fatty acids [10–12], acids [13], aldehydes and alcohols [14], ketones [15], terpenes [16] and some complex carbohydrate derivatives [17]. However, the application of this method to polyunsaturated compounds is not straightforward [18–21], except when the double bonds are separated by several methylene units [22].

One way to apply the DMDS method to polyenes is to carry out first a partial reduction to produce monoenes [23,24]

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followed by DMDS derivative formation and examination by GC–MS. Such strategy was used to examine polyunsaturated fatty acid methyl esters using milligram quantities. However, most pheromone mixtures are available only in nanogram amounts.

We have applied the combined procedure (partial reduction with NH_2NH_2/H_2O_2 and DMDS derivative formation) to determine the complete structure of the major constituent (a triene acetate) of the sex pheromone of *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae) on a 100 ng scale [25–27].

Aiming to improve the partial reduction/DMDS procedure and obtain more information on the partial reduction methods, we have compared the NH_2NH_2/H_2O_2 with NH_2NH_2/O_2 [28,29] (a method used in organic synthesis but not applied to pheromone chemistry). Two synthetic pheromone compounds (9*Z*,11*E*)-9,11-tetradecadienyl acetate [(9*Z*,11*E*)-9,11-TDDA] and (9*Z*,12*E*)-9,12-tetradecadienyl acetate [(9*Z*,12*E*)-9,12-TDDA] were used as models. Later the two partial reduction methods were applied to reduce a total of seven synthetic diene and triene acetates. The partially reduced compounds were reacted with $CH_3–S–S–CH_3/I_2$ to form the DMDS derivatives to locate the double bond positions from their mass spectra. Also, we have described a new "one-pot" procedure where the customary extraction and isolation of monoenes by solvent extraction is not needed before the derivatization by DMDS. In addition, a new procedure is described using ND_2ND_2/H_2O_2 and DMDS derivative formation capable of distinguishing between the double bonds in (*Z*)-9-tetradecenyl acetate and (9*Z*,12*E*)-9,12-tetradecadienyl acetate (1:1 mixture).

2. Materials and methods

2.1. Reagents

Hydrazine hydrate (99%, Mallinckrodt Chemical Co., Pittsburgh, PA), hydrogen peroxide (Fisher Chemical Co., Fair Lawn, NJ) 30% (v/v in water), ND_2ND_2 (99%, MSD Isotopes, Canada), (*Z*)-9-tetradecenyl acetate [(*Z*)-9-TDA], (*E*)-11-tetradecenyl acetate [(*E*)-11-TDA], (*E*)-12-tetradecenyl acetate [(*E*)-12-TDA], (9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-tetradecadienyl acetate [(9*E*,12*Z*)-9,12-TDDA] and (3*E*,13*Z*)-3,13-octadecadienyl acetate [(3*E*,13*Z*)-3,13-ODDA] and dimethyl disulfide (Aldrich Chemical Co., Milwaukee, WI) were purchased from commercial sources. Dr. S. Voerman, Research Institute for Plant Protection, The Netherlands, kindly donated the twenty-three tetradecenyl acetate standards. (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA], (4*Z*,7*Z*,10*Z*)-4,7,10-tetradecatrienyl acetate [(4*Z*,7*Z*,10*Z*)-4,7,10-TDTA] and (5*Z*,8*Z*,11*Z*)-5,8,11-tetradecatrienyl acetate [(5*Z*,8*Z*,11*Z*)-5,8,11-TDTA] were synthesized or available in our laboratory [26].

2.2. Instrumentation

GC analyses were performed using a Hewlett-Packard (HP) 5890 gas chromatograph with FID detection and an integrator. Analyses of the reduced products were carried out on a fused silica capillary column (J&W Scientific, 30 m × 0.22 mm, 0.25 μm film thickness) coated with Carbowax stationary phase. The GC oven was programmed from 60 °C (4 min hold) to 220 °C at 4 °C/min with the injector and detector at 250 and 260 °C, respectively. GC–MS analyses were performed using a Hewlett-Packard (HP) 5890 gas chromatograph coupled to a 5970 Mass Selective Detector or a Finnigan ion trap detector (ITD 800). Both the mass spectrometers were scanned from *m*/*z* 30 to 500 and operated in the electron impact mode (70 eV). For GC–MS analyses of DMDS derivatives, the 30 m × 0.22 mm fused silica capillary column coated with DB-5 stationary phase was used. The GC oven was programmed from 60 °C (3 min hold) to 280 °C at 6 °C/min (20 min hold) with the injector and transfer line at 250 and 280 °C, respectively. Helium was used as the carrier gas for all analyses.

2.3. Partial reduction and DMDS derivative formation

Two partial reduction methods (NH_2NH_2/H_2O_2 and NH_2NH_2/O_2) were evaluated initially on a macro scale (300 μg) at 60 °C using two synthetic pheromone compounds (9*Z*,11*E*)-9,11-TDDA and (9*Z*,12*E*)-9,12-TDDA, as model compounds. After optimization of the reaction conditions, the methodologies were applied for partial reduction of seven synthetic compounds [(9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-TDDA, (3*E*,13*Z*)-3,13-ODDA, (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA] on a micro scale (5 μg). After partial reduction, two methods were used to determine the positions of the remaining double bonds in the monoene acetates. In the first method, the reduced products were isolated by extraction with hexane and reacted with dimethyl disulfide. In the second method ("one-pot method"), the reduced products were not isolated but instead the solvents were evaporated and the residue reacted with dimethyl disulfide. In both cases, the DMDS adducts were analyzed by GC–MS to locate the position of the double bonds.

In order to optimize the reaction conditions, the two model compounds were reacted with four concentrations each of NH_2NH_2 (4, 10, 15 and 20% in methanol) and H_2O_2 (2, 4, 8 and 15% in methanol) using four reaction times (1, 2, 3 and 4 h) at 60 °C. Only one factor was varied at a time. Initially, only the NH_2NH_2 concentration was varied. The relative % of the four products (unreacted diene acetate, completely reduced diene acetate and two monoene acetates) was determined by GC, by dividing the area of each peak by the sum of the four peaks and multiplying by 100. After determining the optimum NH_2NH_2 concentration, the optimum H_2O_2 concentration and the best reaction times were similarly determined. In all cases, the optimum

results were those in which the four compounds were produced.

For the NH_2NH_2/O_2 method, the optimum NH_2NH_2 concentration already obtained with the NH_2NH_2/H_2O_2 method was used along with 30 mg of $CuSO_4{\cdot}5H_2O$ in 3 ml of methanol. Two oxygen flow rates (0.07 and 0.1 ml/min) were evaluated to obtain the optimum response.

The following five methods were used in this study.

2.3.1. Method 1: macro-scale partial reduction with NH_2NH_2/H_2O_2

Both (9*Z*,11*E*)-9,11-TDDA and (9*Z*,12*E*)-9,12-TDDA (300 μg) were mixed separately with the internal standard decyl acetate (DA) (60 μg), and with 3 ml each of NH_2NH_2 (10% in methanol) and H_2O_2 (4% in methanol) in a test tube. The capped test tube was heated at 60 °C for 2 h. After cooling to room temperature, the reaction was quenched with 1 ml of HCl (30% in water) and extracted two hexane portions (1 ml each). The combined organic extract was washed with water (1 ml), dried over anhydrous $MgSO_4$, evaporated to dryness under N_2, redissolved in 300 μl of hexane and 1 μl was analyzed by GC or GC–MS. Peaks were identified by comparing their retention times (t_r) with those of standards as well as from their mass spectra.

2.3.2. Method 2: macro-scale partial reduction with NH_2NH_2/O_2

The reduction and identification were carried out as described in Section 2.3.1 except that H_2O_2 was replaced by 3 ml of methanol containing 30 mg of $CuSO_4.5H_2O$ and a gentle stream of oxygen was passed through the solution at a rate of approximately 0.07 ml/min via a fused-silica capillary (10 cm × 0.22 mm).

2.3.3. Method 3: micro-scale partial reduction of diene and triene acetates with NH_2NH_2/H_2O_2, NH_2NH_2/O_2 and isolation of the reduced compounds followed by DMDS derivatization

Partial reduction was carried out as described above in Sections 2.3.1 and 2.3.2 except 5 μg of the four diene acetates [(9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-TDDA and (3*E*,13*Z*)-3,13-ODDA] and three triene acetates [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA] and 150 μl each of NH_2NH_2 (10% in methanol) and H_2O_2 (4% in methanol) or 150 μl of NH_2NH_2 (10% in methanol) containing 3 mg $CuSO_4.5H_2O$ and a gentle stream of oxygen. Reaction was quenched with100 μl of HCl (30% in water) and the partially reduced products extracted twice with 50 μl hexane portions and analyzed by GC or GC–MS (1 μl). To the remaining solution were added CH_3–S–S–CH_3 (20 μl) and I_2 in ether (20 μl) and left overnight. Excess iodine was reduced with $Na_2S_2O_3$, extracted with two 20 μl hexane portions and 1 μl was analyzed by GC–MS. Peaks after reduction and thiomethylation were identified by comparing their t_r with those of standards as well as from their mass spectra [25–27].

2.3.4. Method 4: "one-pot" micro-scale random reduction of diene and triene acetates with NH_2NH_2/H_2O_2 and NH_2NH_2/O_2 followed by DMDS derivatization (without isolation of reduced products)

These reactions and product identification were conducted as described in Section 2.3.3 with 5 μg of the synthetic diene and triene acetates, except that after partial reduction, about 10% of the total was separated for GC and GC–MS analyses. The rest of the solution was evaporated under N_2 and derivatized with CH_3-S–S–CH_3, as described in Section 2.3.3.

For the sake of convenience, reactions were performed on a 5 μg scale but random reduction/DMDS could be carried out successfully even with 100 ng.

2.3.5. Method 5: micro-scale partial reduction of 1:1 mixture of (9*Z*,12*E*)-9,12-TDDA and (*Z*)-9-TDA with ND_2ND_2/H_2O_2 and isolation of the reduced compounds followed by DMDS derivatization

The reaction was conducted as described in Section 2.3.1 using ND_2ND_2/H_2O_2 in CH_3OD instead of NH_2NH_2/H_2O_2 and a 5 h reaction time. The DMDS derivative was prepared as described in Section 2.3.3. The two deuterated monoene acetates (*Z*)-9-[12,13-$^2H_2$]-TDA and (*E*)-12-[9,10-$^2H_2$]-TDA and their corresponding DMDS derivatives were identified by GC–MS by comparing their mass spectra with those of the corresponding non-deuterated acetates. In the case of the deuterated monoene acetates, the major diagnostic ion utilized was at *m*/*z* 196 (M^+–CH_3COOH), while for the DMDS derivatives the major diagnostic ion used was the molecular ion at *m*/*z* 350. The corresponding diagnostic ions for the non-deuterated monoene acetates and the DMDS derivatives were at *m*/*z* 194 and 348, respectively. No attempt was made to determine the % deuterium incorporation.

3. Statistical analysis

The Microsoft Excel program was used to calculate the averages and standard deviations of the percentages of the reduction products.

4. Results and discussion

Direct reaction of *S. absoluta* pheromone extract with dimethyl disulfide led to the identification of the DMDS derivatives of [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA] and (3*E*,8*Z*)-3,8-tetradecadienyl acetate [21]. However, this procedure for direct identification of double bonds in triene acetates did not work well in our hands. The three pure individual triene acetates (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA on reaction

with dimethyl disulfide produced several peaks on GC–MS analysis. The mass spectra were difficult to interpret. Several reaction conditions were evaluated without success. On the other hand, DMDS derivatives of compounds with one double bond give very characteristic mass spectra [7–17,25–27], easy to interpret. Hence, the strategy to locate double bonds in diene and triene acetates by partial reduction to produce compounds with one double bond followed by DMDS derivative [25–27] was further evaluated in this study.

To carry out the proposed strategy of partial reduction efficiently, followed by DMDS derivatization to locate double bond positions in resulting monoenes, the reaction conditions for the partial reduction step must be optimized to yield high amounts of monoenes. Ideally, a diene (or triene) should be converted entirely into the two (or three) corresponding monoenes. However, this was not possible with either reduction method studied but instead a mixture was obtained, containing the two (or three) desired monoenes, some completely reduced product and some unreacted starting material.

Several concentrations of NH_2NH_2, H_2O_2 and O_2 (different flow rates) and different reaction times were evaluated to optimize the partial reduction of (9*Z*,11*E*)-9,11-TDDA and (9*Z*,12*E*)-9,12-TDDA. The relative proportions of the products obtained is presented in Table 1 [(9*Z*,12*E*)-9,12-TDDA data not presented].

Fig. 1 depicts a section of the reconstructed gas chromatogram (Carbowax stationary phase) obtained on analysis of products from the reaction between (9*Z*,11*E*)-9,11-TDDA and NH_2NH_2/H_2O_2 for 2 h at 60 °C. The reaction yields were reproducible (Table 1). As it can be seen, the chromatogram contained five expected peaks, which were essentially base line resolved. Peak 1 was the internal standard while peaks 2, 3, 4 and 5 were identified as TA (tetradecyl acetate) (*Z*)-9-TDA, (*E*)-11-TDA and unreacted (9*Z*, 11*E*)-9, 11-TDDA, respectively. TA was obtained from complete reduction of (9*Z*,11*E*)-9,11-TDDA) while (*Z*)-9-TDA and (*E*)-11-TDA from the partial reduction of (9*Z*,11*E*)-9,11-TDDA.

The NH_2NH_2/O_2 method produced the same four compounds along with small amounts of isomerized products

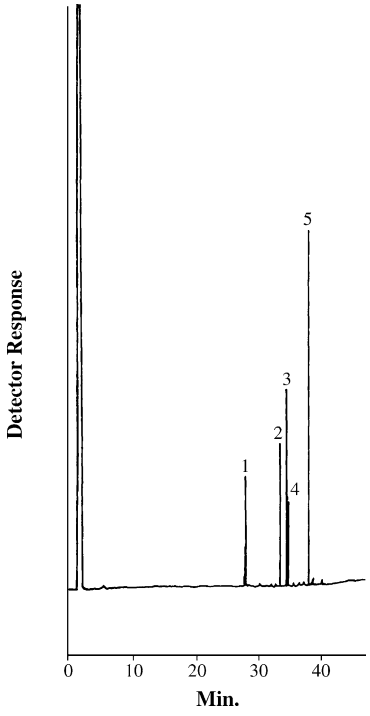


Fig. 1. Typical gas chromatogram obtained on analysis of products obtained by reacting (9*Z*,11*E*)-9,11-tetradecadienyl acetate [(9*Z*,11*E*)-TDDA] with NH_2NH_2/H_2O_2 for 2 h at 60 °C (30 m × 0.22 mm fused silica capillary column coated with Carbowax stationary phase); GC temperature programming: 60 °C for 4 min, 4 °C/min to 220 °C; injector and detector, temperatures 250 and 260 °C, respectively). Numbers 1, 2, 3, 4 and 5 in the chromatogram represent peaks of decyl acetate (DA) (internal standard), tetradecyl acetate (TDA), (*Z*)-11-TDA, (*E*)-11-TDA and (9*Z*,11*E*)-TDDA, respectively.

of the starting diene. Although the degree of isomerization increased gradually as the reaction time increased, the expected reduced products were always the major compounds.

The relative rate of reduction of the *E* and *Z* double bonds depended on the diene acetate used in this study. As expected, the conjugated compound (9*Z*,11*E*)-9,11-TDDA reacted slower than the non-conjugated compound (9*Z*,12*E*)-9,12-TDDA (data not shown) with both the reducing reagents

Table 1
Relative % of products[a] obtained on random reduction of (9*Z*,11*E*)-9,11-tetradecadienyl acetate [(9*Z*,11*E*)-9,11-TDDA] with NH_2NH_2/H_2O_2 (method 1) and NH_2NH_2/O_2 (method 2), along with the ratio of monoenes

Reaction time (h)	Random reduction method	Relative % of				[(*Z*)-9-TDA]/[(*E*)-11-TDA]
		Tetradecyl acetate	(*Z*)-9-TDA	(*E*)-11-TDA	(9*Z*, 11*E*)-9,11-TDDA	
1	1	9.0 ± 0.5	21.5 ± 1.0	9.2 ± 0.2	60.5 ± 1.3	2.34
1	2	2.6 ± 0.2	11.7 ± 0.3	7.2 ± 0.5	78.4 ± 0.7	1.62
2	1	17.5 ± 0.5	24.5 ± 0.5	10.9 ± 0.2	47.0 ± 0.16	2.26
2	2	8.0 ± 1.9	18.00 ± 1.2	10.6 ± 1.2	63.4 ± 4.3	1.70
3	1	27.7 ± 0.6	26.2 ± 0.1	10.4 ± 0.37	36.1 ± 0.2	2.51
3	2	11.9 ± 0.4	18.0 ± 0.2	11.5 ± 0.7	58.6 ± 0.6	1.56
4	1	33.7 ± 1.7	26.4 ± 0.7	10.2 ± 0.3	29.9 ± 0.9	2.59
4	2	13.6 ± 0.5	22.2 ± 0.3	12.8 ± 0.6	51.4 ± 1.0	1.73

[a] Average of three determinations ± standard deviation.

(Table 1). The *Z*/*E* ratio varied slightly from 2.26 to 2.59 with NH_2NH_2/H_2O_2. With NH_2NH_2/O_2, the *Z*/*E* ratio also varied slightly from 1.56 to 1.73. The results of multiple studies on random reduction of different types of compounds with diimide have been compiled [29] and it was reported that the *E* double bond reacted faster than the *Z* double bond.

After the methods were optimized with NH_2NH_2/H_2O_2 and NH_2NH_2/O_2, they were applied to partially reduce the four diene acetates [(9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-TDDA and (3*E*,13*Z*)-3,13-ODDA] and the three triene acetates [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA]. In the case of the diene acetates, four compounds (completely reduced starting material, two monoene acetates and the unreacted starting material) were produced and in the case of triene acetate, eight compounds (completely reduced starting material, three monoene acetates, three diene acetates and unreacted starting material). The reactions were straightforward and consisted of expected compounds only (Figs. 1 and 2).

The partial mass spectra [*m*/*z* (%)] data of all the monoenes obtained from the dienes and trienes after reduction are presented in Table 2. The mass spectra were typical of linear long chain acetates with the base peak at *m*/*z* 43 (CH_3CO^+) in most cases, and a moderately intense ion (∼6%) at *m*/*z* 194 (M^+-CH_3COOH) with no molecular ions being recorded. In addition, the mass spectra were characterized by ions at intervals of 14 units. In these cluster, ions corresponding to C_nH_{2n-1} and C_nH_{2n} were more intense than the C_nH_{2n+1} ions. Although the acetates of a given series presented similar

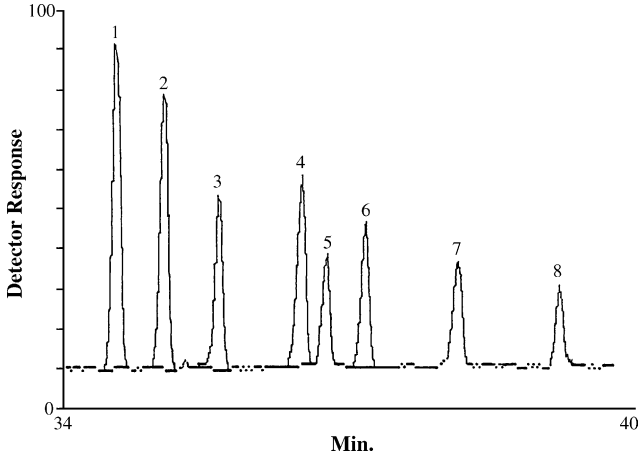


Fig. 2. A section of the reconstructed gas chromatograph obtained on GC–MS analysis of products obtained on partial reduction of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA] with NH_2NH_2/H_2O_2 for 2.5 h at 60 °C on a fused silica capillary column coated with the Carbowax stationary phase. Peaks 1, 2, 3, 4 and 8 are tetradecyl acetate, (*E*)-3-tetradecenyl acetate, (*Z*)-8-tetradecenyl acetate, (*Z*)-11-tetradecenyl acetate and (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, respectively. Peaks 5, 6 and 7 are unidentified isomers of tetradecadienyl acetate.

Table 2
Partial mass spectral data [*m*/*z* (%)] of the monoene acetates obtained on partial reduction of diene* and triene acetates** with NH_2NH_2/H_2O_2 for 2.5 h

Compound	M^+-60	*m*/*z* (%)									
		41	43	54	55	67	68	81	82	95	96
(*Z*)-9-TDA[a]	194 (6.3)	72.6	100	42.0	84.9	50.0	28.9	45.9	45.4	25.5	30.0
(*E*)-11-TDA[a]	194 (5.8)	98.6	100	47.0	67.4	47.6	29.0	49.7	53.5	29.8	26.4
(*Z*)-9-TDA[b]	194 (5.8)	75.0	100	43.2	87.2	51.0	25.0	47.2	53.1	25.9	28.0
(*E*)-12-TDA[b]	194 (6.4)	64.5	98.5	53.0	100	41.3	70.2	33.7	45.4	22.7	29.4
(*E*)-9-TDA[c]	194 (7.3)	70.4	100	38.0	95.5	54.4	28.9	49.7	49.7	29.8	34.8
(*Z*)-12-TDA[c]	194 (6.2)	68.4	100	34.5	88.8	39.9	66.3	32.2	44.5	20.8	21.3
(*E*)-3-ODA[d]	250 (5.2)	37.9	100	63.4	39.4	38.0	51.6	26.3	33.0	16.0	28.9
(*Z*)-13-ODA[d]	250 (6.3)	66.0	90.7	49.0	100	45.2	28.0	40.8	51.1	27.8	42.0
(*E*)-3-TDA[e] (Fig 3A)	194 (5.9)	37.4	100	56.4	33.6	36.3	36.0	24.4	27.8	14.1	23.7
(*Z*)-8-TDA[e] (Fig. 3B)	194 (6.4)	72.0	100	45.5	72.5	55.7	29.9	48.5	48.0	28.7	31.2
(*Z*)-11-TDA[e] (Fig. 3C)	194 (5.7)	97.2	100	29.7	75.6	50.8	60.3	36.9	56.4	24.3	29.2
(*Z*)-4-TDA[f]	194 (8.2)	53.2	100	30.6	35.6	44.4	70.2	39.1	35.3	16.3	20.6
(*Z*)-7-TDA[f]	194 (5.8)	63.6	100	39.3	56.6	55.4	26.3	41.5	42.4	26.0	29.2
(*Z*)-10-TDA[f]	194 (6.4)	56.6	100	40.4	45.9	58.0	28.9	22.9	39.0	35.8	16.9
(*Z*)-5-TDA[g]	194 (5.9)	55.9	100	43.2	43.5	50.9	34.7	34.0	46.2	26.2	27.2
(*Z*)-8-TDA[g]	194 (5.0)	70	100	48.1	81.1	63.0	30.0	47.9	43.0	24.2	30.0
(*Z*)-11-TDA[g]	194 (6.4)	91	100	35.1	81.0	44.2	59.0	35.1	66.0	61.0	21.3

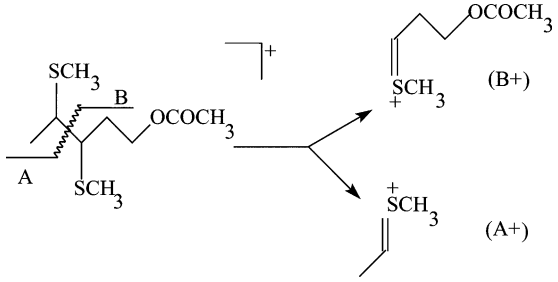
*(9*Z*,11*E*)-9,11-tetradecadienyl acetate [(9*Z*,11*E*)-9,11-TDDA], (9*Z*,12*E*)-9,12-tetradecadienyl acetate [(9*Z*,12*E*)-9,12-TDDA], (9*E*,12*Z*)-9,12-tetradecadienyl acetate [(9*E*,12*Z*)-9,12-TDDA], and (3*E*,13*Z*)-3,13-octadecadienyl acetate [(3*E*,13*Z*)-3,13-ODDA].
**(3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA], (4*Z*,7*Z*,10*Z*)-4,7,10-tetradecatrienyl acetate [(4*Z*,7*Z*,10*Z*)-4,7,10-TDTA] and (5*Z*,8*Z*,11*Z*)-5,8,11-tetradecatrienyl acetate [(5*Z*,8*Z*,11*Z*)-5,8,11-TDTA].
Monoene acetates a, b, c, d, e, f and g were obtained from partial reduction of (9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-TDDA, (3*E*,13*Z*)-3,13-ODDA, (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA, respectively.

mass spectra, the relative proportion of the ions was unique for each acetate. These data were very similar to our previous reports [25–27]. Also, the mass spectra of a given monoene acetate, obtained from partial reduction of two dienes were quite similar, i.e., the mass spectra of (*Z*)-9-TDA obtained from the partial reduction of (9*Z*,11*E*)-9,11-TDDA or (9*Z*,12*E*)-9,12-TDDA were quite similar. The % relative intensities of the geometric isomers (obtained from different compounds) was apparently different, e.g., (*Z*)-9-TDA/(*E*)-9-TDA and (*Z*)-11-TDA/(*E*)-11-TDA.

Thus, it can be concluded that both the partial reduction methods (NH_2NH_2/H_2O_2 and NH_2NH_2/O_2) worked adequately for the reduction of dienes and trienes producing all the expected monoene acetates and could be extended to reduce polyenes. The reducing reagent NH_2NH_2/H_2O_2 was more convenient to use than NH_2NH_2/O_2.

After reduction, two procedures were applied to locate the remaining double bonds in reaction mixture by DMDS derivative formation. The first method involved isolation of the mixture of reduced products by extraction with hexane, followed by DMDS derivative formation. In the second procedure ("one-pot") solvent was evaporated and the reduced products were treated with CH_3–S–S–CH_3/I_2. Although several undesired products (not identified) were formed with both methods, it was possible to locate the DMDS derivatives of the reduced monounsaturated acetates easily. While the "one-pot" procedure was more convenient than the extraction method, it did not provide any other significant advantage over the extraction method.

The partial mass spectra [*m*/*z* (%)] data of all the DMDS derivatives of monoenes generated in this study are presented in Table 3. Most of the DMDS derivatives presented strong molecular ions besides typical fragment ions (A^+ and B^+) by cleavage of the carbon–carbon bond between the two CH_3S groups, as shown below:



In addition, several ions typical of the DMDS derivatives of acetates [9,25–27] corresponding to CH_3CO^+, $CH_3COOH_2^+$ and M^+-CH_3SH were also recorded. Also, the mass spectra of the DMDS derivative of a given monoene acetate, obtained from partial reduction of two dienes were

Table 3
Partial mass spectral data [*m*/*z* (%)] of the DMDS derivatives of monoene acetates obtained on partial reduction of diene* and triene acetates** with NH_2NH_2/H_2O_2 for 2.5 h followed by reaction with CH_3SSCH_3/I_2

DMDS derivative of	*m*/*z* (%)					
	M^+	B^+	A^+	B^+-CH_3COOH	$CH_3COOH_2^+$	CH_3CO^+
(*Z*)-9-TDA[a]	348 (16.3)	231 (39.1)	117 (36.1)	–	61 (15.2)	43 (100)
(*E*)-11-TDA[a]	348 (20.3)	259 (41.2)	89 (35.9)	–	61 (16.8)	43 (100)
(*Z*)-9-TDA[b]	348 (20.2)	231 (33.4)	117 (41.2)	–	61 (18.3)	43 (100)
(*E*)-12-TDA[b]	348 (27.2)	273 (38.1)	75 (43.0)	–	61 (20.3)	43 (100)
(*E*)-9-TDA[c]	348 (24.9)	231 (43.1)	117 (36.1)	–	61 (30.1)	43 (100)
(*Z*)-12-TDA[c]	348 (36.9)	273 (56.0)	75 (34.3)	–	61 (21.9)	43 (100)
(*E*)-3-ODA[d]	404 (20.8)	147 (2.9)	257 (44.0)		61 (24.0)	43 (100)
(*Z*)-13-ODA[d]	404 (31.4)	343 (38.4)	61 (37.1)	–	61 (20.1)	43 (100)
(*E*)-3-TDA[e] (Fig. 4A)	348 (30.3)	147 (0)	201 (78.6)	87(52.6)	61 (40.9)	43 (100)
(*Z*)-8-TDA[e] (Fig. 4B)	348 (41.0)	217 (37.5)	131 (38.0)	–	61 (58.0)	43 (100)
(*Z*)-11-TDA[e] (Fig. 4C)	348 (28.6)	259 (73.2)	89 (36.6)	–	61 (42.8)	43 (100)
(*Z*)-4-TDA[f]	348 (27.3)	161 (10.0)	187 (24.9)	101(14.9)	61 (24.2)	43 (100)
(*Z*)-7-TDA[f]	348 (41.0)	203 (19.9)	145 (71.0)	–	61 (12.8)	43 (100)
(*Z*)-10-TDA[f]	348 (30.2)	245 (14.3)	103 (63.4)	–	61 (15.1)	43 (100)
(*Z*)-5-TDA[g]	348 (21.3)	175 (38.2)	173 (69.0)	–	61 (13.1)	43 (100)
(*Z*)-8-TDA[g]	348 (29.2)	217 (14.3)	131 (79.0)	–	61 (31.2)	43 (100)
(*Z*)-11-TDA[g]	348 (30.3)	259 (31.1)	89 (41.0)	–	61 (23.1)	43 (100)

*(9*Z*,11*E*)-9,11-tetradecadienyl acetate [(9*Z*,11*E*)-9,11-TDDA], (9*Z*,12*E*)-9,12-tetradecadienyl acetate [(9*Z*,12*E*)-9,12-TDDA], (9*E*,12*Z*)-9,12-tetradecadienyl acetate [(9*E*,12*Z*)-9,12-TDDA], and (3*E*,13*Z*)-3,13-octadecadienyl acetate [(3*E*,13*Z*)-3,13-ODDA].
**(3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA], (4*Z*,7*Z*,10*Z*)-4,7,10-tetradecatrienyl acetate [(4*Z*,7*Z*,10*Z*)-4,7,10-TDTA] and (5*Z*,8*Z*,11*Z*)-5,8,11-tetradecatrienyl acetate [(5*Z*,8*Z*,11*Z*)-5,8,11-TDTA].
Monoene acetates a, b, c, d, e, f and g were obtained from partial reduction of (9*Z*,11*E*)-9,11-TDDA, (9*Z*,12*E*)-9,12-TDDA, (9*E*,12*Z*)-9,12-TDDA, (3*E*,13*Z*)-3,13-ODDA, (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, (4*Z*,7*Z*,10*Z*)-4,7,10-TDTA and (5*Z*,8*Z*,11*Z*)-5,8,11-TDTA, respectively followed by reaction with CH_3SSCH_3/I_2.

quite similar, i.e., the mass spectra of DMDS derivative of (*Z*)-9-TDA obtained from the partial reduction/thiomethylation of (9*Z*,11*E*)-9,11-TDDA or (9*Z*,12*E*)-9,12-TDDA were quite similar.

The reduction/thiomethylation procedure used to determine the positions of the double bonds in trienes is illustrated with (3*E*,8*Z*,11*Z*)-3,8,11-TDTA. The triene acetate was first randomly reduced to obtain three monoene acetates (along with other compounds), which were identified by GC–MS. The position of double bond in each of the three acetates was further confirmed by DMDS derivative formation, which in turn determined the positions of the double bonds in (3*E*,8*Z*,11*Z*)-3,8,11-TDTA.

A section of the reconstructed gas chromatogram obtained on partial reduction of (3*E*,8*Z*,11*Z*)-3,8,11-TDTA (100 ng) with NH_2NH_2/H_2O_2 for 2.5 h at 60 °C is presented in Fig. 2. Eight compounds were expected and detected. Peaks 1, 2, 3, 4 and 8 were identified as TA, (*E*)-3-tetradecenyl acetate [(*E*)-3-TDA], (*Z*)-8-tetradecenyl acetate [(*Z*)-8-TDA] and (*Z*)-11-tetradecenyl acetate [(*Z*)-11-TDA] and (3*E*,8*Z*,11*Z*)-3,8,11-TDTA, respectively (the mass spectra of the three monoene acetates are presented in Fig. 3). The remaining three peaks, 5–7 were identified as isomers of tetradecadienyl acetates. However, it was not possible to identify either the positions of the double bonds or their geometry since the standards were not available.

TA was obtained from complete reduction of (3*E*,8*Z*,11*Z*)-3,8,11-TDTA while the three monoene and three diene acetates were obtained from partial reduction. The three monoene acetates could be separated on capillary columns coated with the non-polar stationary phase (DB-1) or on a phase of intermediate polarity (Carbowax). The latter phase allowed a base line separation of the eight compounds. The elution of the compounds from the GC column coated with Carbowax was in increasing order of polarity of the compounds, i.e., saturated compound, monoene acetates, diene acetates and triene acetate.

Thus, having identified the three monoene acetates by GC–MS, the next step was to confirm the position and geometry of the double bond in the three acetates by DMDS formation. The three DMDS derived from the three monoenes after partial reduction were base line resolved on the capillary column coated with the DB-1 stationary phase. The background subtracted mass spectra of the three DMDS derivatives are presented in Fig. 4 with the mass spectral data presented in Table 3. The three DMDS derivatives presented strong molecular ions. The DMDS derivative of (*E*)-3-TDA (Fig. 4A) did not present the two expected fragment ions at *m*/*z* 201 and 147 but instead exhibited the ions at *m*/*z* 201 (78.6%) and 87 (52.6%). The ion at *m*/*z* 87was derived from the fragment ion at *m*/*z* 147 with the loss of CH_3COOH as shown:

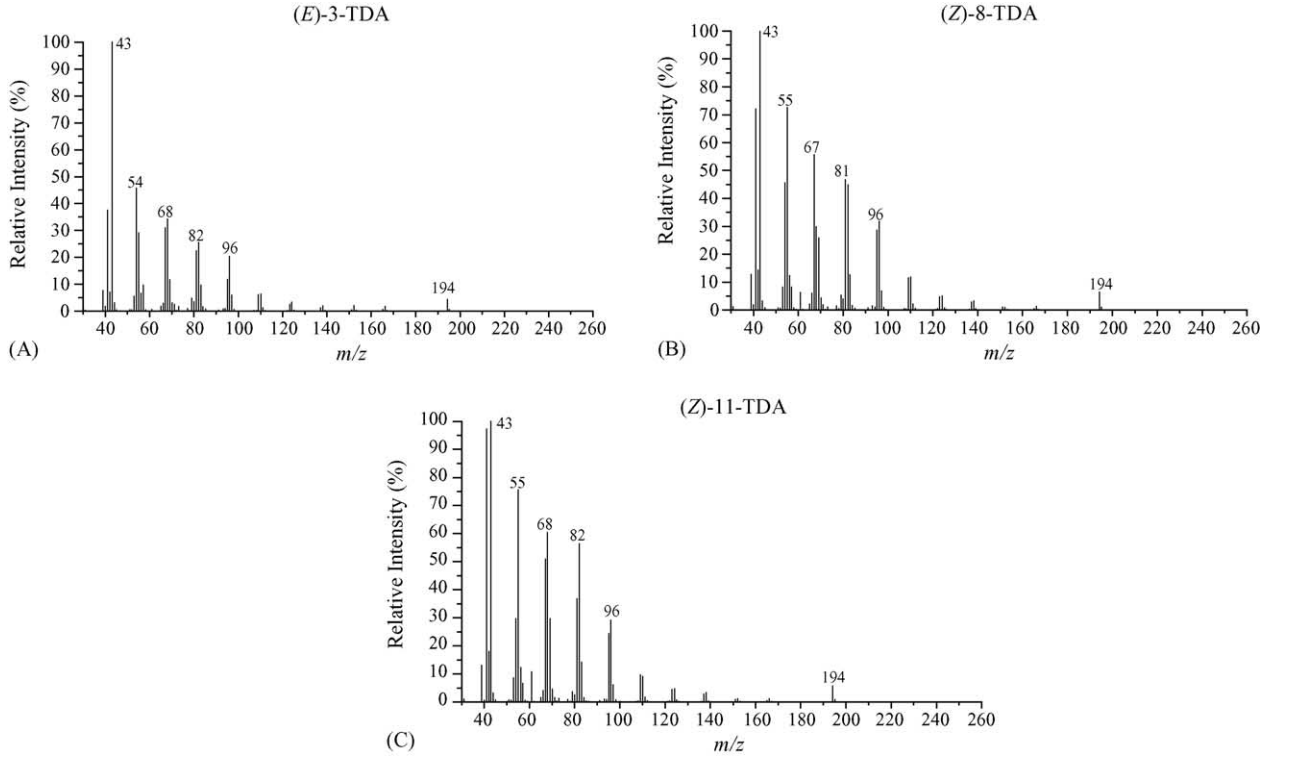
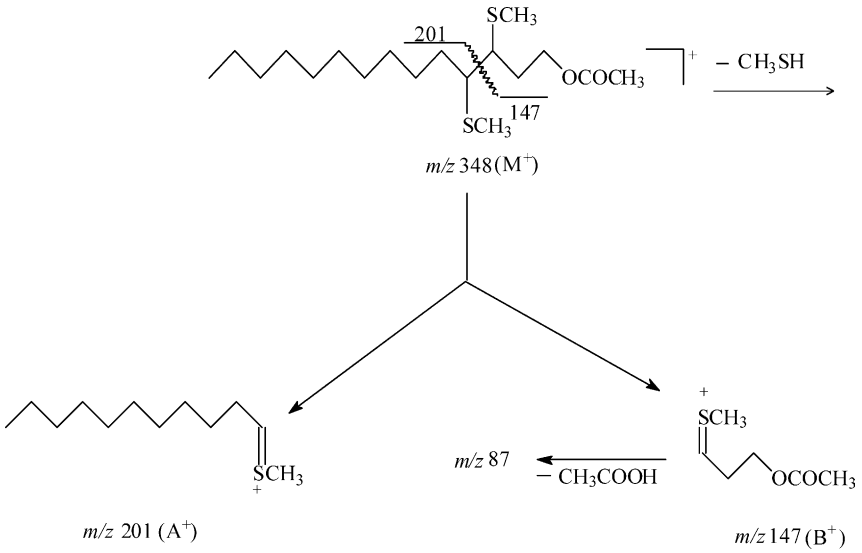


Fig. 3. Background subtracted mass spectra of peaks 2, 3 and 4 (Fig. 2) identified as (*E*)-3-tetradecenyl acetate (A), (*Z*)-8-tetradecenyl acetate (B) and (*Z*)-11-tetradecenyl acetate (C).



The DMDS derivative of (*Z*)-8-TDA exhibited the two fragment ions at *m*/*z* 217 (37.5%) and 131 (38%) (Fig. 4B). The DMDS derivative of (*Z*)-11-TDA also exhibited the two typical fragment ions at *m*/*z* 259 (73.2%) and 89 (36.6%) (Fig. 4C).

The above-described method worked adequately for the four diene acetates and three triene acetates in the absence of significant amounts of monoenes. However, most biological samples are complex mixtures, which often contain monoenes in addition to polyenes. Fortunately, in most cases,

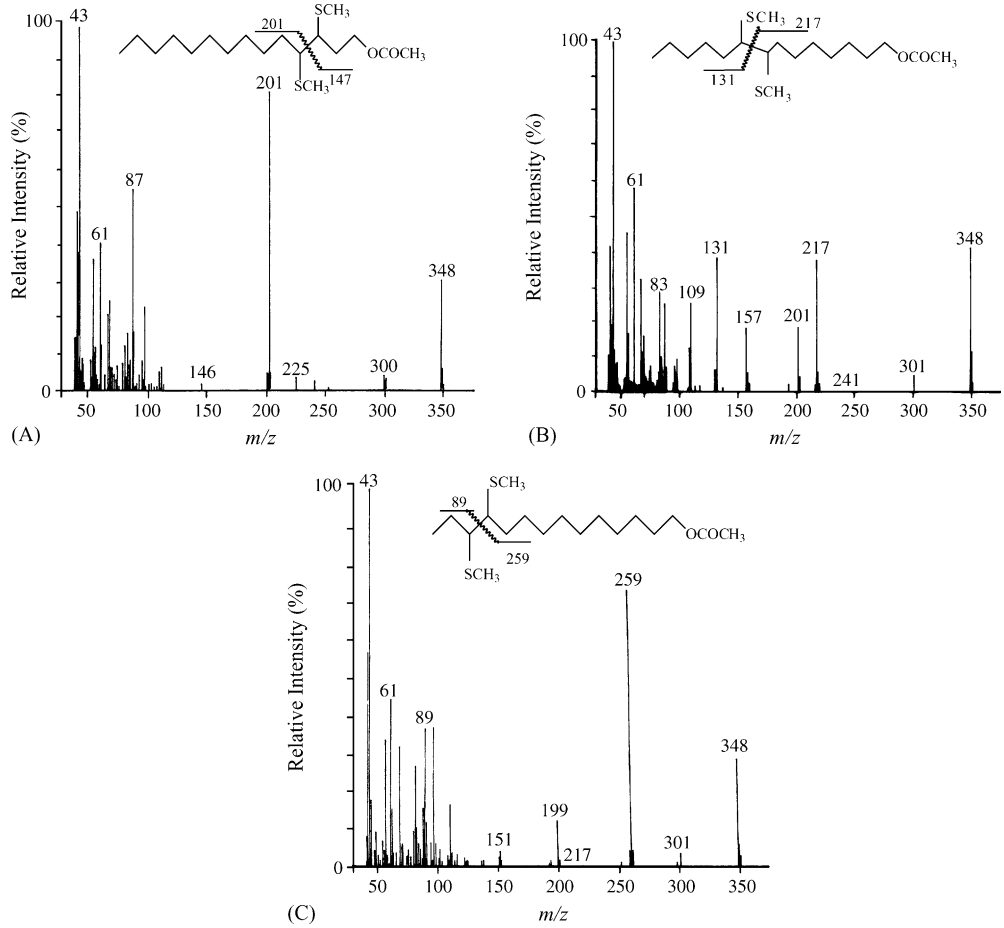


Fig. 4. Background subtracted mass spectra of DMDS derivatives of (*E*)-3-tetradecenyl acetate (A), (*Z*)-8-tetradecenyl acetate (B) and (*Z*)-11-tetradecenyl acetate (C) obtained on partial reduction of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrienyl acetate [(3*E*,8*Z*,11*Z*)-3,8,11-TDTA] with NH_2NH_2/H_2O_2 for 2.5 h at 60 °C followed by DMDS derivative formation.

these monoene/polyene mixtures arise from similar biosynthetic pathways. Consequently, the positions of double bonds in the monoenes are usually identical to those in the polyenes [30,31]. In such cases, the monoenes produced by random reduction can be recognized even in the presence of other monoenes (produced from partial reduction of other polyenes) if ND_2ND_2 is used instead of NH_2NH_2. Such a distinction would be possible only when mixtures of monoenes and dienes are present. However, exceptions must not be precluded.

To validate the above method, we reduced a 1:1 mixture of (*Z*)-9-TDA and (9*Z*,12*E*)-9,12-TDDA with ND_2ND_2/H_2O_2 followed by DMDS derivative formation. The compounds identified after deuteration are presented in Fig. 5, with the following three monoene acetates being detected on GC–MS analysis (after reduction): (*Z*)-9-TDA (unreacted), (*Z*)-9-

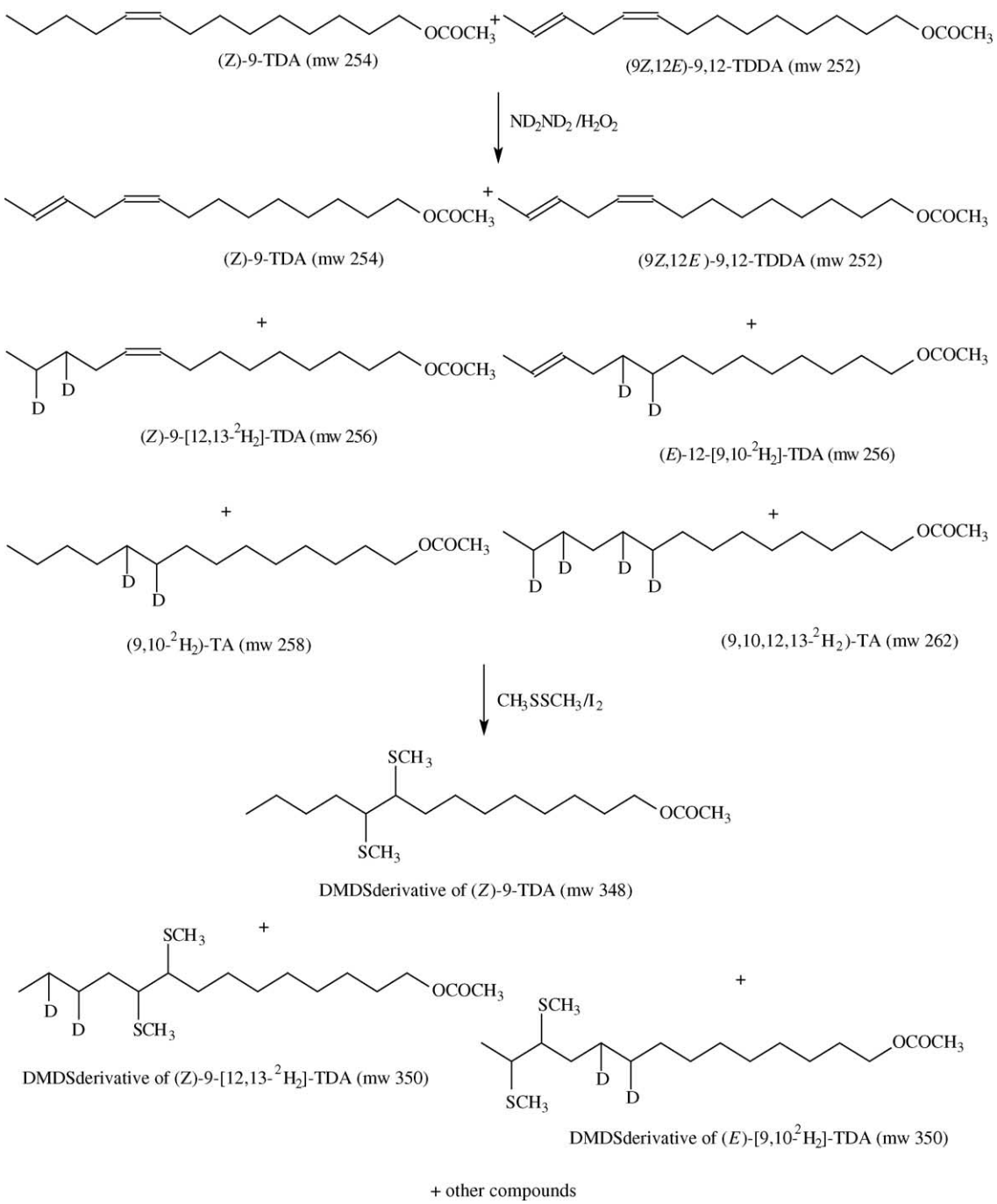


Fig. 5. Compounds obtained on reduction of a 1:1 mixture of (*Z*)-9-TDA and (9*Z*,12*E*)-9,12-TDDA (1:1) with ND_2ND_2/H_2O_2 in CH_3OD for 5 h at 60 °C, extraction and DMDS derivative formation.

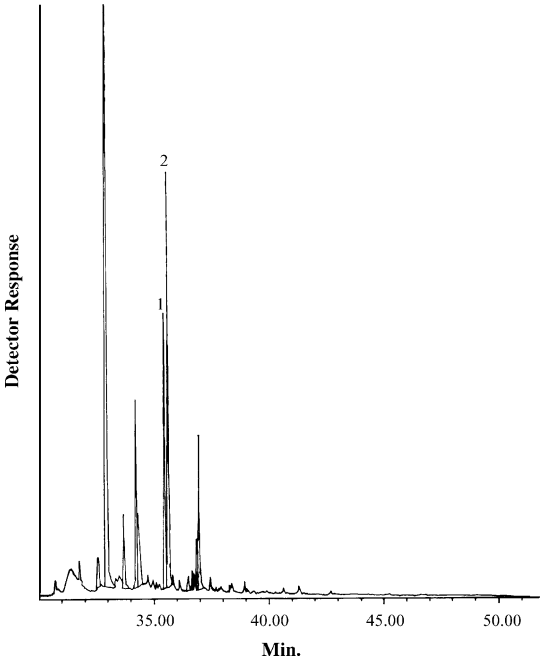


Fig. 6. A section of the reconstructed gas chromatogram obtained on GC–MS analysis of products obtained by reacting a 1:1 mixture of (*Z*)-9-TDA and (9*Z*,12*E*)-9,12-TDDA (1:1) with ND_2ND_2/H_2O_2 in CH_3OD for 5 h at 60 °C, extraction and DMDS derivative formation (30 m × 0.22 mm fused silica capillary column coated with DB-5 stationary phase); GC temperature programming: 60 °C for 3 min, 6 °C/min to 280 °C (20 min. hold); injector and transfer line temperatures 250 and 280 °C, respectively. Peaks 1 and 2 represent the DMDS derivatives of (*Z*)-9-TDA + (*Z*)-9-[12,13-$^2H_2$]-TDA and (*E*)-12-[9,10-$^2H_2$]-TDA, respectively.

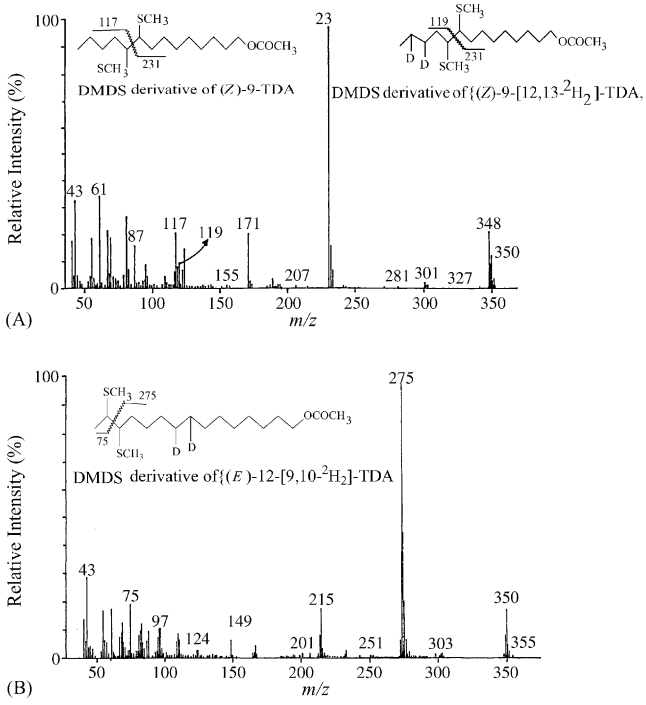


Fig. 7. Background subtracted mass spectra of peaks 1 {DMDS derivative of (*Z*)-9-TDA and (*Z*)-9-[12,13-$^2H_2$]-TDA} (A) and peak 2 (DMDS derivative of {(*E*)-12-[9,10-$^2H_2$]-TDA} (B) (see Fig. 6 for definition of peaks 1 and 2).

[12,13-$^2H_2$]-TDA and (*E*)-12-[9,10-$^2H_2$]-TDA. Peaks 1 and 2 (Fig. 6) were identified as the DMDS derivatives of (*Z*)-9-TDA+(*Z*)-9-[12,13-$^2H_2$]-TDA and (*E*)-12-[9,10-$^2H_2$]-TDA, respectively.

The background subtracted mass spectra of the three DMDS derivatives are presented in Fig. 7 along with partial mass spectral data in Table 4. The mixed mass spectrum of the DMDS derivatives of (*Z*)-9-TDA and (*Z*)-9-[12,13-$^2H_2$]-TDA (Fig. 7A) presented the expected fragment ions from each of the two compounds. Thus, the ions at *m*/*z* 350 (M^+, 12.5%) and the two fragment ions at *m*/*z* 231 (100%) and 119 (9.7%) were produced from (*Z*)-9-[12,13-$^2H_2$]-TDA while the ions at *m*/*z* 348 (M^+, 21.6%) 231 (100%) and 117 (20.5%) from (*Z*)-9-TDA. The DMDS derivative of (*E*)-12-[9,10-$^2H_2$]-TDA eluted as a single peak from the GC column (Fig. 7B). It presented ions at *m*/*z* 350 (M^+, 17.0%) and the two fragment ions at *m*/*z* 275 (100%) and 75 (19.3%) (Fig. 7B). The fragment at *m*/*z* contained the two deuterium atoms. The corresponding ions in the non-deuterated compound were at *m*/*z* 273 and 75, respectively. In addition, several ions typical of the DMDS derivatives of acetates [9,25–27] corresponding toCH_3CO^+, $CH_3COOH_2^+$ and M^+-CH_3SH were also recorded. Selective ion monitoring data was consistent with above fragmentation and GC elution.

5. Conclusions

None of the partial reduction methods evaluated in this study could produce the monoenes predominantly. The DMDS derivatization step after reduction was not straightforward since a very complex mixture was formed, limiting

Table 4
Partial mass spectral data [*m*/*z* (%)] of the DMDS derivatives of monoene acetates obtained on partial reduction of a 1:1 mixture of (*Z*)-9-TDA and (9*Z*,12*E*)-9,12-TDDA (1:1) with ND_2ND_2/H_2O_2 in CH_3OD for 5 h at 60 °C by reaction with CH_3SSCH_3/I_2

DMDS derivative of	*m*/*z* (%)					
	M^+	B^+	A^+	B^+-CH_3COOH	$CH_3COOH_2^+$	CH_3CO^+
(*Z*)-9-TDA[a] (Fig. 7A)	348 (21.6)	231 (100)	117 (20.5)	171 (20.5)	61 (35.2)	43 (34.1)
(*Z*)-9-[12,13-$^2H_2$]-TDA[a] (Fig. 7A)	350 (12.5)	231 (100)	119 (9.7)	171 (20.5)	61 (35.2)	43 (34.1)
(*E*)-12-[9,10-$^2H_2$]-TDA (Fig. 7B)	350 (17.0)	275 (100)	75 (19.3)	215 (17.4)	61 (17.0)	43 (29.5)

[a] Data obtained from mixed mass spectra of (*Z*)-9-TDA and (*Z*)-9-[12,13-$^2H_2$]-TDA as the compounds could not be resolved on GC–MS analysis (Fig. 7).

the sensitivity of the combined reduction/DMDS procedure. More selective reduction methods should be investigated for polyene reduction. Although none of the reduction methods offered a significant advantage over the other, reduction with NH_2NH_2/H_2O_2 was more convenient hence it should be the method of choice together with DMDS derivative formation to locate double bonds in pheromones. Despite the limitations, the random reduction-DMDS procedure can be applied, in principle, to any polyene (soluble in hexane or other such solvents) to locate the double bonds on a nanogram scale.

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