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Journal of Chromatography A, 1048 (2004) 59–65
JOURNAL OF CHROMATOGRAPHY A
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# Efficient method to locate double bond positions in conjugated trienes

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Received 1 April 2004; received in revised form 8 July 2004; accepted 9 July 2004

Available online 8 August 2004

**Abstract**

The double bond positions of 11 conjugated trienes were unambiguously located through a simple derivatization method amenable to nanogram-scale analyses. The trienes were reacted with the powerful dienophile 4-methyl-1,2,4-triazoline-3,5-dione (MTAD), and the mass spectra of the resulting cycloadducts exhibited large diagnostic fragments which allowed the unequivocal location of the double bonds in the parent triene in most cases. Catalytic hydrogenation of the cycloadducts produced saturated compounds with characteristic mass spectral fragments from which the positions of the trienes in the parent compounds could be readily confirmed. Application of the method was demonstrated by the microscale identification of two conjugated triene and one conjugated diene components from extracts of the sex pheromone gland of the saturniid moth *Automeris cecrops pamina*.


*Keywords:* Conjugated triene; Double bond position determination; 4-Methyl-1,2,4-triazoline-3,5-dione; Hydrogenation

## 1. Introduction

Alkenes are common functional groups in lepidopteran sex pheromones, with the position and stereochemistry of the double bonds being critically important to their biological activity. However, location of double bond positions and determination of their stereochemistries in the nanogram quantities usually available from extracts of the pheromone glands can be challenging.

GC–MS analysis has been the method of choice for beginning the identification of most volatile insect pheromones because of its combination of high resolving power and high sensitivity [1,2]. However, the similarity of mass spectra and the easy migration of double bonds under electron-impact ionization conditions render it difficult to determine double bond positions unequivocally in the long-chain alcohols, aldehydes, and acetates that constitute many lepidopteran pheromones [3]. Derivatization of the compound to produce an adduct from which the double bond position(s) in the parent compound can be deduced from the mass spectrum of the adduct has been employed to solve this problem. Useful derivatization reactions for this purpose have been reviewed [1,2,4], with the most common among them including ozonolysis [5], epoxidation [6,7] and formation of vicinal bis-methylthiol ethers [8,9]. Derivatization reactions have also been developed for use with conjugated dienes, in particular, the Diels–Alder reaction of a conjugated diene with a dienophile such as 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) followed by GC–MS analysis of the resulting cycloadducts [10]. This method has been applied successfully to the identification of sex pheromone components [11] and their precursors [12] for several lepidopteran species, and for the identification of unsaturated hydrocarbons from the defensive secretion of a bombardier beetle, *Metrius contractus* [13].

### 1.1. Conjugated trienes

The complete identification of conjugated trienes from insect pheromone gland extracts, such as 10*E*,12*E*,14*Z*-16:Ald,

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doi:10.1016/j.chroma.2004.07.017

a pheromone component of the sphingid moth *Manduca sexta* [14], can be difficult because of the limited stability of the compounds and the lack of a straightforward method to locate the positions of the double bonds. By modification of a method developed by Vouros and co-workers [10], we have successfully located the double bonds of several conjugated trienes, including some known sex pheromone components and related compounds. Thus, a triene, or a crude extract containing the triene, is reacted with the dienophile (MTAD). In most cases, the position of the triene in the parent molecule can be determined directly from large diagnostic fragments in the mass spectra of the resulting cycloadduct(s). For the minority of cases in which the position of the triene in the parent compound cannot be determined unequivocally at this stage, a second straightforward catalytic hydrogenation step performed on the crude mixture yields the reduced cycloadduct(s). The mass spectra of the reduced adducts exhibit diagnostic fragments from which the position of the triene in the parent compound can be unambiguously located.

## 2. Experimental

The trienes employed in this study were taken from a library of standards assembled during the course of other projects. Specifically, the syntheses of 7,9,11-dodecatriene type compounds are described in [15], 9,11,13-tetradecatriene compounds are described in [16], 9,11,13-hexadecatriene type compounds are described in [17], and 10,12,14-hexadecatriene type compounds are described in [18].

### 2.1. Coupled gas chromatography–mass spectrometry

EI mass spectra (70 eV) were taken with a Hewlett-Packard (HP) 6890 gas chromatograph interfaced to a 5973 mass selective detector. A 30 m × 0.25 mm i.d. HP-5MS column was used, with an injector temperature of 300 °C, and a temperature program of 100 °C/0 min, then 15 °C/min to 300 °C for 20 min. The solvent delay was set at 8 min, and a mass range of 40–500 U was scanned.

### 2.2. Reaction of trienes with MTAD

One μl of a stock solution of the respective triene (1 mg/ml in hexane) was transferred to a Kimble 0.25 ml glass conical vial insert containing 10 μl of $CH_2Cl_2$, and the solution was treated with 10 μl of a 2 mg/ml solution of MTAD in $CH_2Cl_2$. After 15 min at room temperature, the faint pink solution was concentrated under a gentle stream of nitrogen to ∼5 μl, and a 1 μl aliquot was analyzed by splitless GC–MS. For the treatment of extracts of insect pheromone glands, the dissected glands were soaked in pentane for 20 min, then, the extract was transferred to a clean vial. The extract was concentrated under a stream of nitrogen to ∼10 μl, and 1 μl aliquots were analyzed by GC–MS and coupled gas chromatography–electroantennogram detection, allowing the tentative identification of one $C_{16}$ conjugated diene and two $C_{16}$ conjugated trienes, by comparison of retention times and mass spectra with those of standards. The remainder of the extract was treated dropwise with the MTAD solution until the faint pink color of excess MTAD persisted. Because of the small amounts of materials in the extract, the resulting solution was analyzed in selected ion monitoring mode to improve sensitivity, using four ions diagnostic for the MTAD adducts of 10,12,14-triene aldehydes [*m*/*z* 149, 206 (base peak), 319, and 347 (molecular ion)].

### 2.3. Hydrogenation of MTAD cycloadducts

An aliquot of the MTAD cycloadducts prepared as described above was transferred to a 1.5 ml screw-capped glass vial with 200 μl of EtOAc and 0.5 mg of 5% Pd on activated charcoal catalyst. A balloon fitted with a syringe needle was filled with hydrogen and inserted through the PTFE-faced septum sealing the vial, and the mixture was stirred 10–12 h at room temperature. The catalyst was then removed by filtering the sample through a small pad of cellite, and the resulting solution was concentrated under a gentle stream of nitrogen

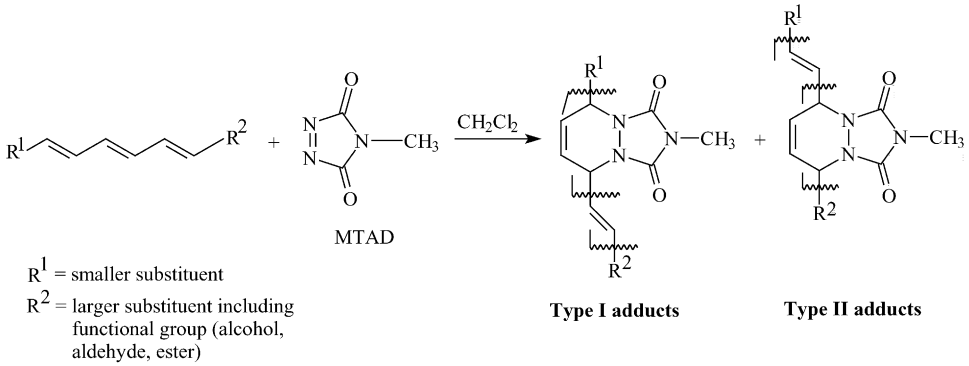


Fig. 1. Regioisomeric cycloadducts obtained from reaction of trienes with MTAD and the key mass spectral fragmentations used to locate the conjugated double bond positions.

to 5 μl. A 1 μl aliquot was analyzed by splitless GC–MS as described above.

## 3. Results and discussion

The two possible cycloaddition products (types I and II) from Diels–Alder reaction of a conjugated triene with MTAD, and the important fragmentations expected in the mass spectra of the adducts, are outlined in Fig. 1.

Table 1 lists the relative abundance of these fragments for a number of alcohol, aldehyde, and ester pheromone components.

The reactions of trienes with MTAD were generally very clean, giving only the cycloadducts as products (e.g., Fig. 2).

Of the four types of functional groups tested, aldehydes gave particularly strong diagnostic fragments, with one of the fragments often being the base peak, whereas, the diagnostic even-mass fragments from esters and alcohols were slightly smaller (~20–50% of base peak; compare Fig. 3A–E).

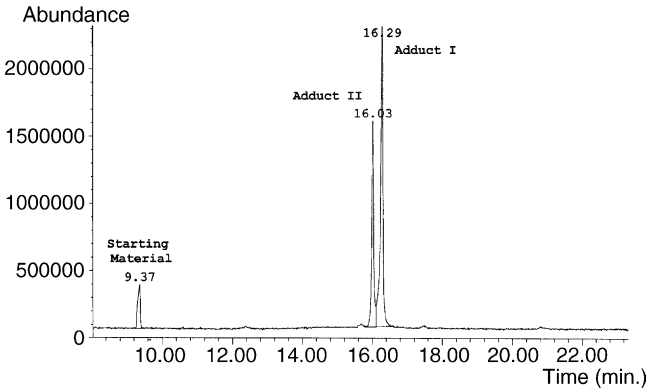


Fig. 2. Total ion chromatogram (GC–MS) obtained from analysis of the reaction of 10*E*,12*E*,14*E*-16:Ald with MTAD.

A number of general points emerged from examination of the mass spectra of the adducts listed in Table 1. First, the MTAD adducts of all compounds tested produced well defined molecular ion peaks, providing useful confirmation of the molecular ions of the parent compounds by subtraction

Table 1
Important fragments in the EI mass spectra of the cycloadducts generated by reaction of trienes with MTAD

| Entry | Triene (adduct type) | Retention time (abundance) | Mass fragments (abundance) | | | | |
|---|---|---|---|---|---|---|---|
| | | | $M^+$ | $[M\text{-}R_1]^+$ | $[M\text{-}(R_1 + CH\overline{=}CH)]^+$ | $[M\text{-}R_2]^+$ | $[M\text{-}(R_2 + CH\overline{=}CH)]^+$ |
| 1 | 10*E*,12*E*,14*E*-16:OH | | | | | | |
| | Type II | 16.62[a] (48) | 349 (17) | 334 (1) | 308 (0) | 206 (100) | 180 (2) |
| | Type I | 16.89[a] (100) | 349 (43) | 334 (20) | 308 (1) | 206 (88) | 180 (30) |
| 2 | 9*E*,11*E*,13-14:OH | | | | | | |
| | Type II | 15.08[a] (100) | 321 (14) | 320 (0) | 294 (1) | 192 (100) | 166 (1) |
| | Type I | 15.99[a] (37) | 321 (59) | 320 (0) | 294 (0) | 192 (62) | 166 (36) |
| 3 | 10*E*,12*E*,14*E*-16:Ald | | | | | | |
| | Type II | 16.03 (58) | 347 (10) | 332 (1) | 306 (1) | 206 (100) | 180 (1) |
| | Type I | 16.29 (100) | 347 (27) | 332 (14) | 306 (1) | 206 (68) | 180 (25) |
| 4 | 7*Z*,9*E*,11-12:formate | | | | | | |
| | Type I | 13.94 | 321 (50) | N/A | N/A | 192 (25) | 166 (31) |
| 5 | 7*Z*,9*E*,11-12:OH | | | | | | |
| | Type I | 14.48[a] | 293 (23) | N/A | N/A | 192 (19) | 166 (41) |
| 6 | 7*Z*,9*E*,11-12:OAc | | | | | | |
| | Type I | 14.34 | 335 (43) | N/A | N/A | 192 (18) | 166 (29) |
| 7 | 9*Z*,11*E*,13-14:Ald | | | | | | |
| | Type I | 14.63 | 319 (17) | N/A | N/A | 192 (16) | 166 (44) |
| 8 | 10*E*,12*E*,14*Z*-16:Ald | | | | | | |
| | Type II | 16.24[a] | 347 (7) | 332 (1) | 306 (1) | 206 (100) | 180 (1) |
| 9 | 9*E*,11*E*/*Z*,13*E*-16:OH | | | | | | |
| | Type II | 16.27[a] (98) | 349 (19) | 320 (7) | 294 (1) | 220 (100) | 194 (1) |
| | Type I | 16.74[a] (100) | 349 (25) | 320 (100) | 294 (1) | 220 (39) | 194 (16) |
| 10 | 9*E*,11*Z*,13*E*-16:OAc | | | | | | |
| | Type II | 16.04 (100) | 391 (18) | 362 (12) | 336 (2) | 220 (100) | 194 (2) |
| | Type I | 16.54 (93) | 391 (46) | 362 (91) | 336 (0) | 220 (100) | 194 (24) |
| 11 | 9*E*,11*Z*,13*Z*-16:Ald | | | | | | |
| | Type I | 14.85 (100) | 347 (17) | 318 (100) | 292 (1) | 220 (20) | 194 (6) |
| | Type I | 15.04 (28) | 347 (21) | 318 (100) | 292 (1) | 220 (20) | 194 (8) |

[a] Retention time measured in a new column.

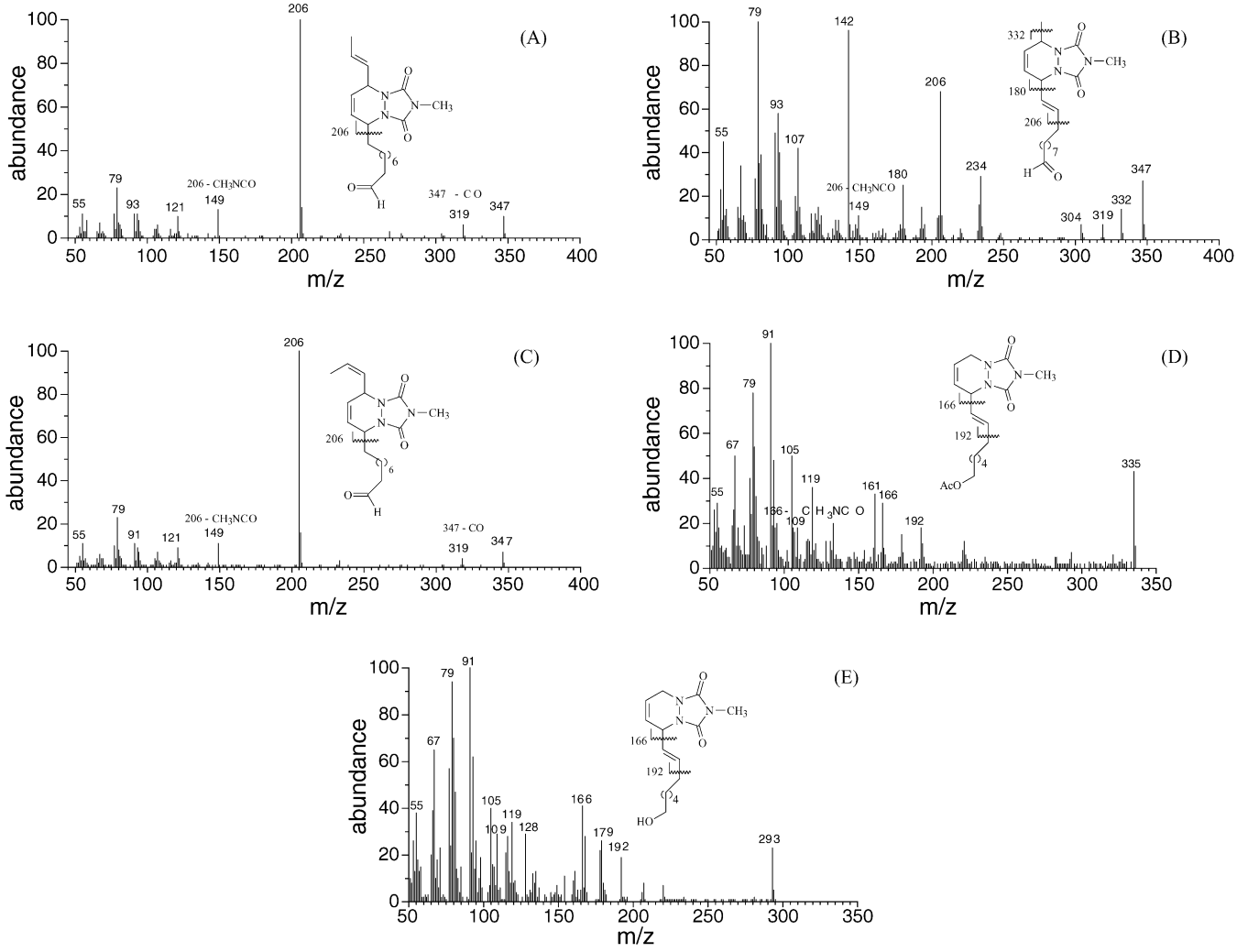


Fig. 3. Electron impact mass spectra (70 eV) of MTAD adducts of 10*E*,12*E*,14*E*-16:Ald (A) and (B); 10*E*,12*E*,14*Z*-16:Ald (C); 7*Z*,9*E*,11-12:OAc (D); and 7*Z*,9*E*, 11-12:OH (E).

of 113 U, the molecular mass of MTAD, from the $M^+$ ion of the adduct. Second, for each parent compound, two regioisomeric adducts were theoretically possible, but in practice, the ratio of the two regioisomers was dependent upon the steric congestion in the transition state of the Diels–Alder reaction. Thus, compounds with three 1,2-disubstituted *E* double bonds (Table 1, entries 1 and 3) gave an approximately 2:1 ratio of adducts of types I and II (Fig. 1). The single compound with two *E* double bonds and a terminal double bond also gave two adducts, with the ratio of adducts favoring type II (Table 1, entry 2). In contrast, compounds with one *Z* and one *E* and a terminal double bond produced adducts exclusively from reaction of the terminal butadiene unit (Table 1, entries 4–7). 10*E*,12*E*,14*Z*-16:Ald also produced only a single type II adduct from reaction of the 10*E*,12*E*-diene unit (Table 1, entry 8), demonstrating the effect of steric congestion about the 14*Z* double bond. For the compound having two *Z* double bonds (Table 1, entry 11), the cycloaddition reaction was markedly slower, with unreacted starting material being detected upon analysis of the solutions of the trienes and MTAD. Thus, the number of adducts obtained, and their ratios, may provide some information about the possible stereochemistries of the double bonds.

Third, in all cases, the type II adducts gave very simple mass spectra dominated by a base peak from favored cleavage of the large, saturated alkyl group alpha to the nitrogen (e.g., *m*/*z* 206 ion in Fig. 3A and C). In contrast, the type I adducts gave more complex spectra, with significant ions from loss of $R^1$, $R^2$, and $R^2CH{=}CH$ fragments (Table 1 and Fig. 3B; *m*/*z* 332, 206, and 180, respectively). The loss of $R^2$ may occur after migration of the exocyclic alkene into conjugation with the endocyclic double bond, leading to an allylic rather than a vinylic fragmentation.

Furthermore, the ion from loss of $R^2CH{=}CH$ in the type I adduct was 26 U smaller than the base peak in the corresponding type II adduct, showing the link between the two structures differing only in the positioning of the adduct along the triene. For most of the cases studied, type I adducts also

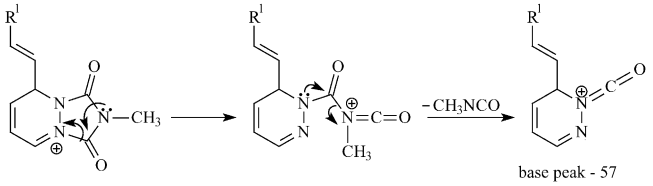


Fig. 4. Postulated fragmentation leading to the loss of methyl isocyanate from the base peak.

produced a significant $[R^2 + H]^+$ ion (*m/z* 142 in Fig. 3B). Thus, the combination of the data from the types I and II adducts usually allowed the unequivocal placement of the triene, for example, in the 10,12,14-position for the adducts shown in Fig. 3A and B.

For those compounds that yielded only a single adduct, it may still be possible to deduce the position of the triene in the parent compound from a combination of the parent molecule's molecular weight and the fragmentation of the adduct. For example, the three analogous compounds 7*Z*,9*E*,11-12:OCHO, –OH, and –OAc (Table 1, entries 4–6) all produced significant even-numbered fragments at *m/z* 192 and 166 from losses of $R^2$ and $R^2CH{=}CH$, respectively. In this case, the *m/z* 166 ion can only arise from $R^1{=}H$, confirming that the conjugated triene is terminal. It may not be possible to determine the triene position exactly for other compounds that give only a single adduct because the ions from cleavage of $R^2$ from adducts of types I or II cannot be distinguished. The problem may be further compounded with aldehydes, where $R^1$ and $R^2$ fragments may have the same nominal masses (e.g., $C_5H_{11}$ and $C_4H_7O$). However, careful examination of the spectrum may resolve this conundrum because spectra of type II adducts are dominated by the single large even-mass ion from the highly favored hydrogen transfer and loss of $R^2$, whereas the spectrum from a type I adduct contains several significant even-mass ions. Thus, determination of whether the spectrum is that of a types I or II adduct, followed by assignment of the diagnostic fragments, allows the triene position to be ascertained.

Two other fragment ions are worth noting. First, the adducts from aldehydes (Table 1, entries 3, 7, 8, 11) all produced a small but distinct ion at $[M\text{-}28]^+$. This ion was probably from loss of CO from the aldehyde function because

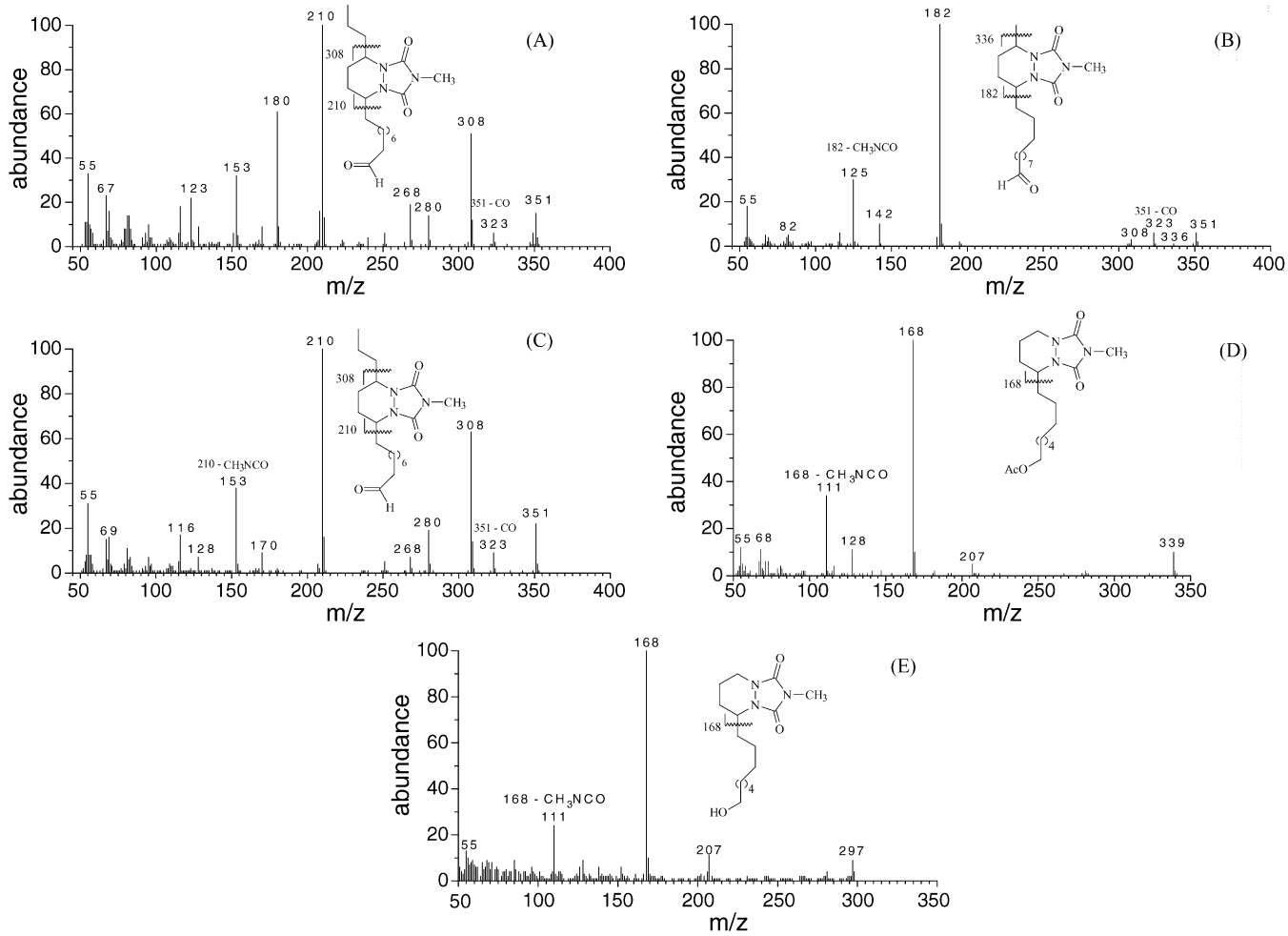


Fig. 5. Electron-impact mass spectra (70 eV) of hydrogenated MTAD adducts of 10*E*,12*E*,14*E*-16:Ald (A) and (B); 10*E*,12*E*,14*Z*-16:Ald (C); 7*Z*,9*E*,11-12:OAc (D); and 7*Z*,9*E*,11-12:OH (E).

Table 2
Important fragments in the EI mass spectra of the hydrogenated cycloadducts generated by reaction of trienes with MTAD

| Entry | Triene (adduct type) | Retention time (abundance) | Mass fragments (abundance) | | |
|---|---|---|---|---|---|
| | | | $M^+$ | $M^+$-$R_3$ | $M^+$-$R_4$ |
| 1 | 10*E*,12*E*,14*E*-16:OH | | | | |
| | Type II derivative | 17.33[a] (59) | 353 (33) | 310 (35) | 210 (100) |
| | Type I derivative | 18.18[a] (100) | 353 (20) | 338 (1) | 182 (100) |
| 2 | 9*E*,11*E*,13-14:OH | | | | |
| | Type II derivative | 15.55[a] (100) | 325 (25) | 296 (26) | 196 (100) |
| | Type I derivative | 16.79[a] (53) | 325 (18) | 324 (0) | 168 (100) |
| 3 | 10*E*,12*E*,14*E*-16:Ald | | | | |
| | Type II derivative | 16.51 (64) | 351 (15) | 308 (51) | 210 (100) |
| | Type I derivative | 17.15 (100) | 351 (6) | 323 (6) | 182 (100) |
| 4 | 7*Z*,9*E*,11-12:formate | | | | |
| | Type I derivative | 14.37 | 325 (25) | 324 (0) | 168 (100) |
| 5 | 7*Z*,9*E*,11-12:OH | | | | |
| | Type I derivative | 14.91[a] | 297 (9) | 296 (2) | 168 (100) |
| 6 | 7*Z*,9*E*,11-12:OAc | | | | |
| | Type I derivative | 14.87 | 339 (10) | 338 (0) | 168 (100) |
| 7 | 9*Z*,11*E*,13-14:Ald | | | | |
| | Type I derivative | 15.06 | 323 (10) | 322 (0) | 168 (100) |
| 8 | 10*E*,12*E*,14*Z*-16:Ald | | | | |
| | Type II derivative | 15.35[a] | 351 (22) | 308 (63) | 210 (100) |
| 9 | 9*E*,11*Z*/*E*,13*E*-16:OH | | | | |
| | Type II derivative | 16.97 (100) | 353 (33) | 296 (44) | 224 (100) |
| | Type I derivative | 17.64 (96) | 353 (21) | 324 (21) | 196 (100) |
| 10 | 9*E*,11*Z*,13*E*-16:OAc | | | | |
| | Type II derivative | 16.82 (34) | 395 (24) | 338 (25) | 224 (100) |
| | Type II derivative | 17.12 (88) | 395 (17) | 338 (30) | 224 (100) |
| | Type I derivative | 17.51 (45) | 395 (14) | 366 (12) | 196 (100) |
| | Type I derivative | 17.71 (100) | 395 (14) | 366 (13) | 196 (100) |
| 11 | 9*E*,11*Z*,13*Z*-16:Ald | | | | |
| | Type I derivative | 15.68 (62) | 351 (13) | 322 (45) | 196 (100) |
| | Type I derivative | 15.82 (100) | 351 (14) | 322 (50) | 196 (100) |

[a] Retention time measured in a new column.

this ion was not seen in the spectra from the alcohols or esters. Second, a fragment derived from the loss of methyl isocyanate from the base peak ion [base peak-57]$^+$ was observed (e.g., *m*/*z* 149 in Fig. 3A–C), presumably from a fragmentation such as that shown in Fig. 4.

In general, although the mass spectra of the MTAD cycloadducts from the triene test compounds were more complicated than those from conjugated dienes, it was usually straightforward to locate the double bond positions by careful analysis of the mass spectra of the cycloadducts. To remove any possible doubt as to the positions of the trienes in the parent compounds, hydrogenation of the crude mixtures of the adducts followed by GC–MS of the saturated adducts provided unequivocal confirmation of the double bond positions (Fig. 5 and Table 2).

For example, reaction of 10*E*,12*E*,14*Z*:16:Ald, a component of the sex pheromone blend of the tobacco hornworm *M. sexta* [14] with MTAD produced a single adduct (Table 1, entry 8), which, after hydrogenation, yielded a saturated compound. The base peak in the mass spectrum of the saturated compound, at *m*/*z* 182 from cleavage of $C_{11}H_{21}O^{\cdot}$, Fig. 5B, and a small fragment at *m*/*z* 336 [$M^+$-15], confirm that the conjugated triene in the parent molecule must have been in the 10, 12, 14 positions.

In general, fragmentation alpha to the nitrogen gave abundant fragments, with loss of the larger substituent always being the base peak (Fig. 6).

As a further general trend, it was observed that regioisomers of type II, derived from reaction of MTAD with the more internal double bonds of the trienes, had shorter retention times than adducts of type I. The retention times of the hydrogenated cycloadducts showed a similar trend.

Although the method cannot distinguish geometric isomers (see Fig. 3A and C), some information regarding the stereochemistry of the double bonds can be extracted by carefully analyzing the results. The reaction of 10*E*,12*E*,14*E*-16:Ald with MTAD afforded two cycloadducts (Table 1, entry 3 and Fig. 3A and B), whereas the reaction of its stereoisomer 10*E*,12*E*,14*Z*-16:Ald (Table 1, entry 8 and Fig. 3C) with MTAD afforded only one. This fact is a clear indication that

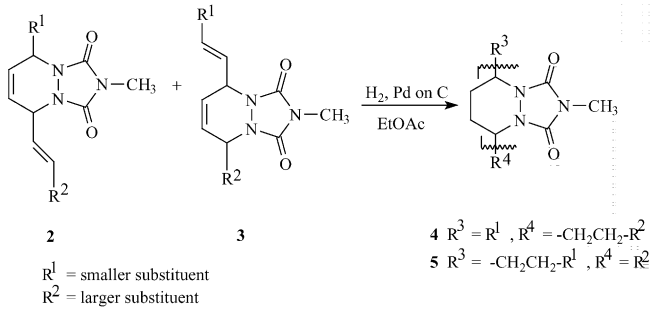


Fig. 6. Diagnostic fragmentations observed in the EI mass spectra of hydrogenated MTAD cycloadducts.

in the first case the energies of the two transition states which led to the formation of the cycloadducts were not very different, while in the second case, the energy of one transition state was much higher than the energy of the other.

During development of the method, the quantities of trienes used ranged from 150 ng to 1 μg. However, to illustrate the application of this methodology to a real-world sample, the method was used to determine the double bond positions of the likely pheromone components in a crude extract of the sex pheromone gland of the saturniid moth *Automeris cecrops pamina* (Neumoegen, 1882) [19]. GC–MS analysis and coupled gas chromatography with electroantennogram detection allowed the tentative identification of a $C_{16}$ conjugated diene aldehyde and two $C_{16}$ conjugated triene aldehydes in the extract. After treatment of the crude extract with MTAD, the conjugated system in the diene component was determined to be in the 10, 12 positions, and the conjugated trienes were determined to be in the 10, 12, 14 positions for both compounds, from the mass spectra of the MTAD adducts. Further comparisons of the retention times and mass spectra of the insect-produced compounds with those of authentic standards conclusively identified the insect produced components as 10*E*,12*Z*-16:Ald, 10*E*,12*E*,14*E*-16:Ald, and 10*E*,12*E*,14*Z*-16:Ald.

In summary, although the mass spectra of the cycloadducts obtained from the reaction of trienes with MTAD were slightly more complicated than the mass spectra from the analogous derivatives of conjugated dienes, it was easily possible to locate the double bond positions after a single very straightforward reaction with MTAD, for most of the trienes tested. If necessary, the even simpler mass spectra of the hydrogenated derivatives could be used to corroborate the double bond positions in the parent conjugated triene compounds.

## Acknowledgements

F.A.M. thanks the Brazilian Council for the Development of Science and Technology (CNPq) for the financial support. The work described was also supported by Hatch Project CA-R*ENT-5181-H to J.G.M.

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