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Gas chromatography–mass spectrometry of conjugated dienes by derivatization with 4-methyl-1,2,4-triazoline-3,5-dione

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ABSTRACT

The dienophile 4-methyl-1,2,4-triazoline-3,5-dione forms stable adducts with conjugated dienes by generating Diels–Alder cycloaddition products. The reaction is rapid, highly selective for conjugated dienes and the derivatives are suitable for analysis by gas chromatography. Their mass spectra are marked by their simplicity and by the presence of abundant fragment ions diagnostic of the diene position in the parent compound.

INTRODUCTION

A wide variety of naturally occurring organic compounds bearing a long carbon chain contains one or more sites of unsaturation. Definition of the position of unsaturation by mass spectrometry has normally involved a chemical modification of the double bond in order to overcome the problems presented by the randomization of the π-system upon ionization [1]. For example, when using chemical ionization mass spectrometry (CI-MS), the double bond may be localized by reaction with iron ions and the subsequent collisional activation leads to fragment ions diagnostic of the double bond position [2]. Other CI reagents that interact specifically with double bonds include methyl vinyl ether [3], and $Ar–O_3–H_2O$ mixtures [4]. Methods involving electron impact ionization require the formation of derivatives which direct the fragmentation of the molecule about the carbons associated with the original double bond. A classical example is the conversion of the alkene to a glycol followed by formation of a trimethylsilyl ether [5] or isopropylidene [6] derivative.

Conjugated dienes, which are important in certain insects as pheromones, semiochemicals or sex attractants [7–9], require alternative approaches. We recently

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reported on the use of the electrophilic reagent, 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), as a selective derivatizing reagent for conjugated dienes [10]. The reagent forms Diels–Alder adducts which exhibit prominent molecular ion peaks and characteristic fragment ions which are diagnostic of the position of the conjugated diene on the chain. The PTAD adducts of conjugated dienes were characterized by a high thermal stability. However, their low volatility limits their general applicability for gas chromatography (GC)–MS analysis. We report here on the preparation and utilization of 4-methyl-1,2,4-triazoline-3,5-dione (MTAD), as a derivatizing reagent for conjugated dienes. In addition to displaying structurally informative mass spectra the MTAD adducts appear to be very well suited for analysis by GC–MS as illustrated by their relatively low Kováts indices and good thermal stability.

EXPERIMENTAL

Synthesis of 4-methyl-1,2,4-triazoline-3,5-dione

The procedure for the synthesis of MTAD was based on the method of Cookson et al. [11] for the preparation of PTAD, with the exception that N_2O_4 instead of *tert.*-butyl hypochlorite was used for oxidation of the corresponding urazole. Milligram quantitities of 4-methylurazole (Aldrich, U.S.A.), were placed in the bottom of a Pyrex culture tube, 1.5 ml methylene chloride was then added and the mixture placed in an ice bath. N_2O_4 was gently blown into the tube until the reddish-brown color of NO_2 was seen to fill the tube. The tube was then capped and shaken until the color in the gas phase was observed to go mostly into solution. The red MTAD color develops in minutes and the reaction is complete when all the urazole is seen to go into solution. (A second portion of N_2O_4 may be necessary to effect the dissolution of larger amounts of urazole). After generating the MTAD, the culture tube was transferred to a bath of warm water and the solution evaporated nearly to dryness with a stream of nitrogen in order to eliminate excess N_2O_4.

Preparation of derivatives of conjugated dienes

MTAD adducts of conjugated dienes were prepared as described previously for the PTAD adducts [9]. Quantities in the range of 500 ng to 1 μg were used to generate the data described in this report. The reaction is nearly instantaneous and occurs at room temperature.

Instrumentation

GC–MS analyses were performed on a JEOL DX303HF double-focusing mass spectrometer equipped with a modified Hewlett-Packard gas chromatograph and a JEOL DA5000 data system run by a Digital PDP 11/73 minicomputer. GC injections were made using the split injection mode. The filament and accelerating potential were turned off for 4 min after the injection to protect the mass spectrometer. The injector was set at 300°C and the initial column temperature was 100°C. After 2 min the column was ramped at 15°C/min to 280°C. A 60-m SE-52 capillary column and an alkane mixture of C_{14}, C_{18}, C_{24} and C_{28} were used to assign retention indices.

RESULTS AND DISCUSSION

Fig. 1 shows the Diels–Alder cycloaddition reaction of MTAD with a conjugated diene. Adducts were formed between MTAD and the dienes shown on Table I which summarizes the Kováts retention indices for the test compounds. By comparison to the corresponding PTAD analogues, the MTAD adducts exhibit a dramatic improvement in volatility. For example, the PTAD adducts of 2*E*,4*Z*-hexadiene and of 9*Z*,11*E*-tetradecadienyl acetate were found to have retention indices

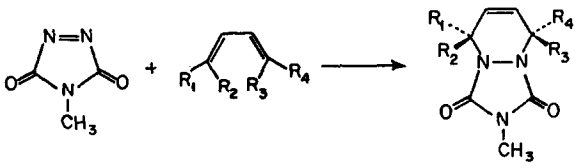

Fig. 1. Cycloaddition reaction of MTAD with a conjugated diene.

of 2210 and 3400, respectively. These compare to values of 1580 and 2750 for the corresponding MTAD derivatives. The chromatographic peak shapes of the MTAD derivatives were comparable to those of the hydrocarbons used for the Kováts index calibrations. Fig. 2A shows a total ion chromatogram of three conjugated diene adducts. An expanded display of the segment encompassing the elution of C_{14} through C_{18} allows a better comparison of the chromatographic behavior of the MTAD adducts with that of the non-polar alkanes.

The MS of the MTAD adducts is fundamentally equivalent to that of the PTAD analogues. The spectra are both simple and easy to interpret. They exhibit well defined molecular ion peaks as well as abundant fragment ions which result from loss of an alkyl side chain and which are diagnostic of the diene position in the hydrocarbon chain. The latter, generally, give rise to the base peak, $[M-R]^+$ and/or $[M-R']^+$, depending on the number of alkyl substituents. This fragmentation is clearly alpha to the nitrogen, typical of the spectra of simple alkyl amines (Fig. 3). A second major fragmentation frequently observed is the loss of methyl isocyanate from the $[M-R]^+$ ions (Fig. 4). This loss is probably favored because of further extension of the

TABLE I

RETENTION INDICES OF THE MTAD DERIVATIVES OF SELECTED CONJUGATED DIENES

Compound		Kováts index
No.	Name	
I	2*E*,4*E*-Hexadiene	1580
II	2*E*,4*Z*-Hexadiene	1580
III	1,3-Hexadiene	1680
IV	7*E*,9*Z*-Dodecadien-1-yl acetate	2530
V	8*E*,10*E*-Dodecadien-1-yl acetate	2560
VI	9*Z*,11*E*-Tetradecadien-1-yl acetate	2750

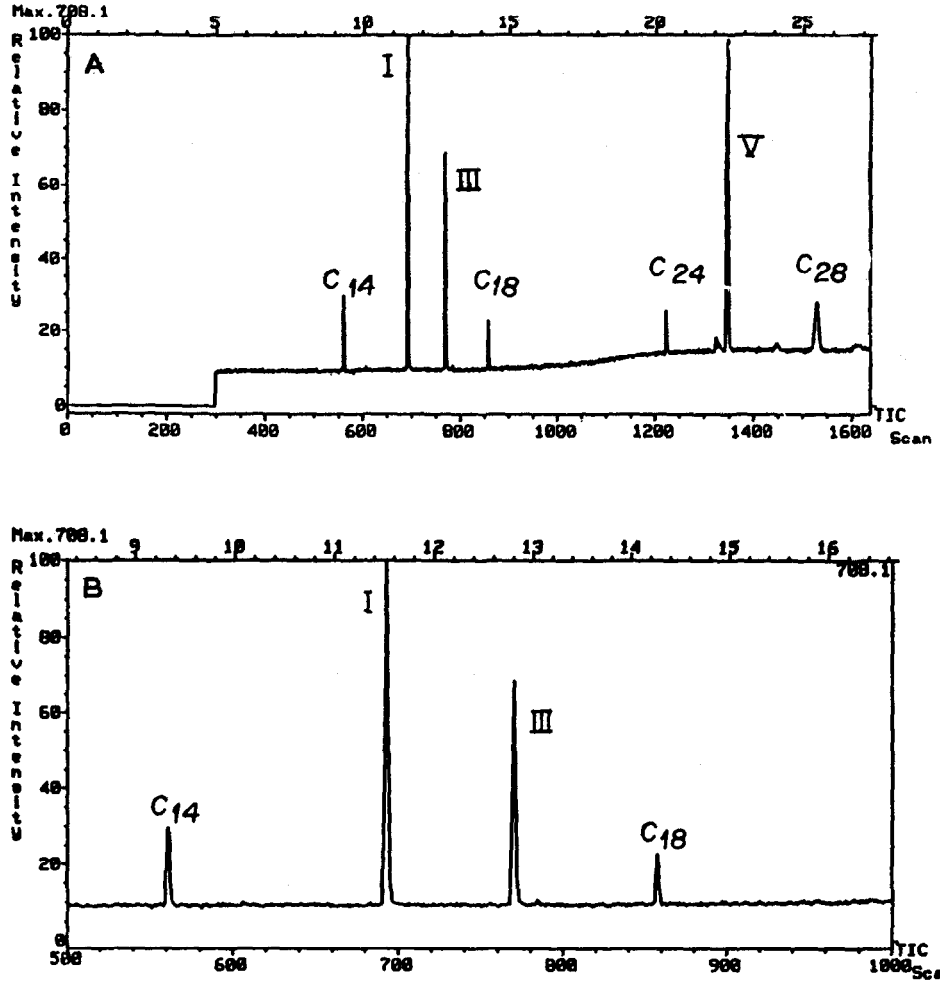

Fig. 2. (A) GC–MS total ion chromatogram of MTAD adducts of 2*E*,4*E*-hexadiene (I), 1,3-hexadiene (III) and 8*E*,10*E*-dodecadien-1-yl-acetate (V), along with indicated alkanes. (b) Expanded segment of C_{14} through C_{18} of Fig. 2A.

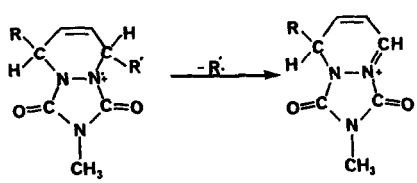

Fig. 3. Probable mechanism for the initial loss of side chain from MTAD–conjugated diene adducts.

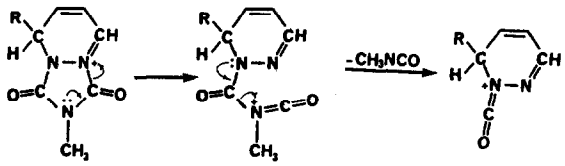

Fig. 4. Mechanism for the loss of methyl isocyanate after formation of the intermediate shown in Fig. 3.

conjugation as well as relief of the potential for instability caused by having the positive iminium center next to a carbonyl carbon in its $[M-R]^+$ precursor.

The mass spectra of three isomeric hexadiene derivatives are compared in Fig. 5. While MTAD adduct formation cannot distinguish between geometric isomers —compare spectra in Figs. 5A and B— positional identification of the conjugated alkenes is readily accomplished, as illustrated from the spectrum in Figs. 5C. The mass spectra of two isomeric dodecadienyl acetates are further compared in Figs. 6A and B. Again, a well defined loss of the side chain in both spectra helps identify the diene positions. The mass spectrum of a tetradecadienyl analogue of the latter two compounds is also shown in Fig. 6, to illustrate the general applicability of the derivatives.

In summary, the mass spectrometry of the MTAD derivatives of conjugated dienes is clearly dominated by the heterocyclic nucleus of the 1,2,4-triazoline-3,5-dione system. The relatively simple spectra resulting from these large, but symmetrical, compounds could probably not be duplicated by another type of derivative and, thus the possibility of error in their structural assignment is minimized. Spectral interpretation is straightforward. All that is necessary is to look for a pair of ions separated by the mass of methyl isocyanate (57 u) and subtract the higher mass ion from the molecular ion in order to determine the mass of one of the groups that was attached to the original conjugated diene. The fact that reaction of MTAD with a conjugated diene results in the formation of only one detectable product in all the cases examined, is ideal behavior for a derivative. Derivatization with 4-methyl-1,2,4-triazoline-3,5-dione should provide a method of choice for the GC–MS analysis of conjugated aliphatic dienes.

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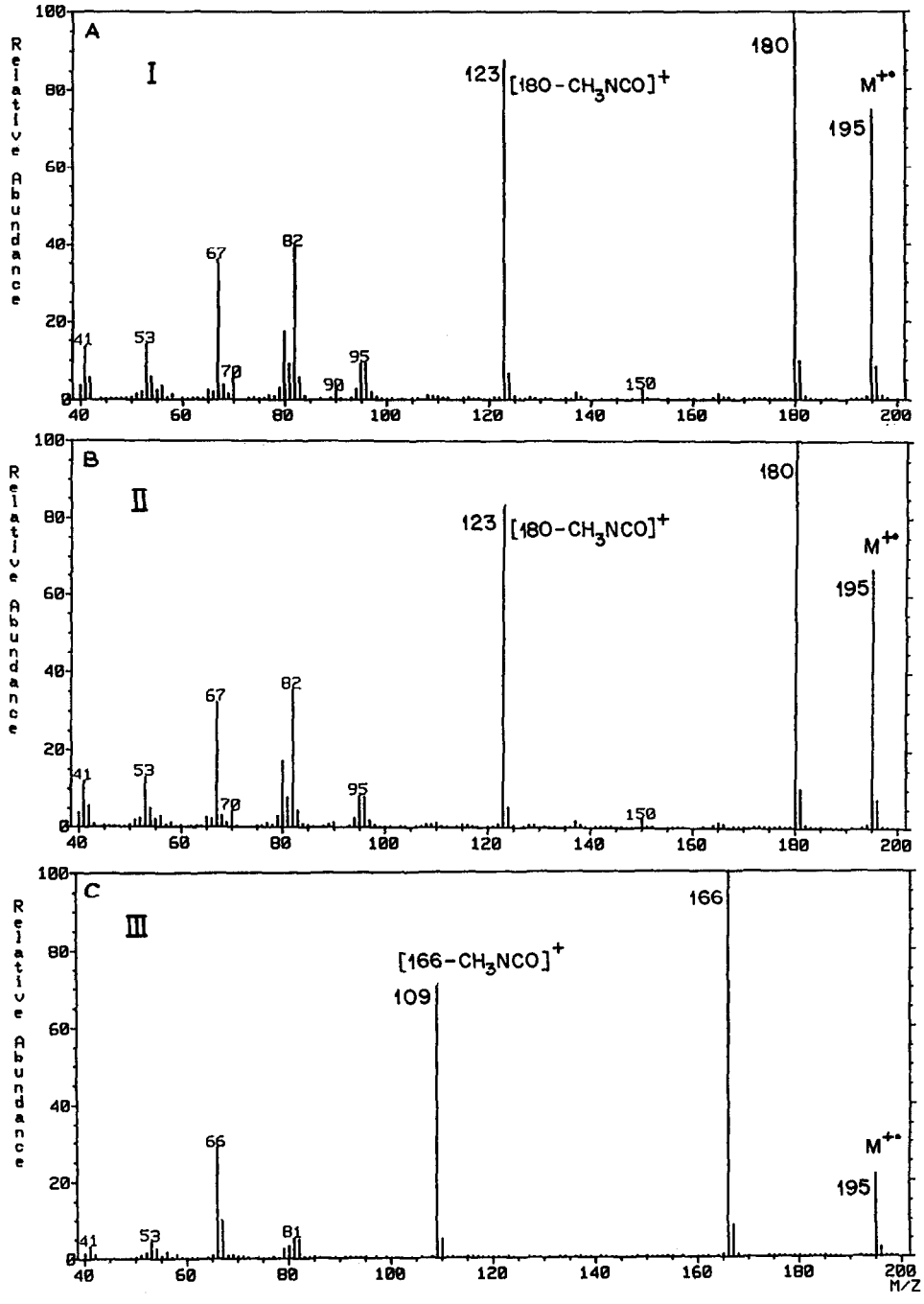

Fig. 5. Electron impact mass spectra of MTAD derivatives of (A) 2*E*,4*E*-hexadiene. (I); (B) 2*E*,4*Z*-hexadiene (II); and (C) 1,3-hexadiene (III).

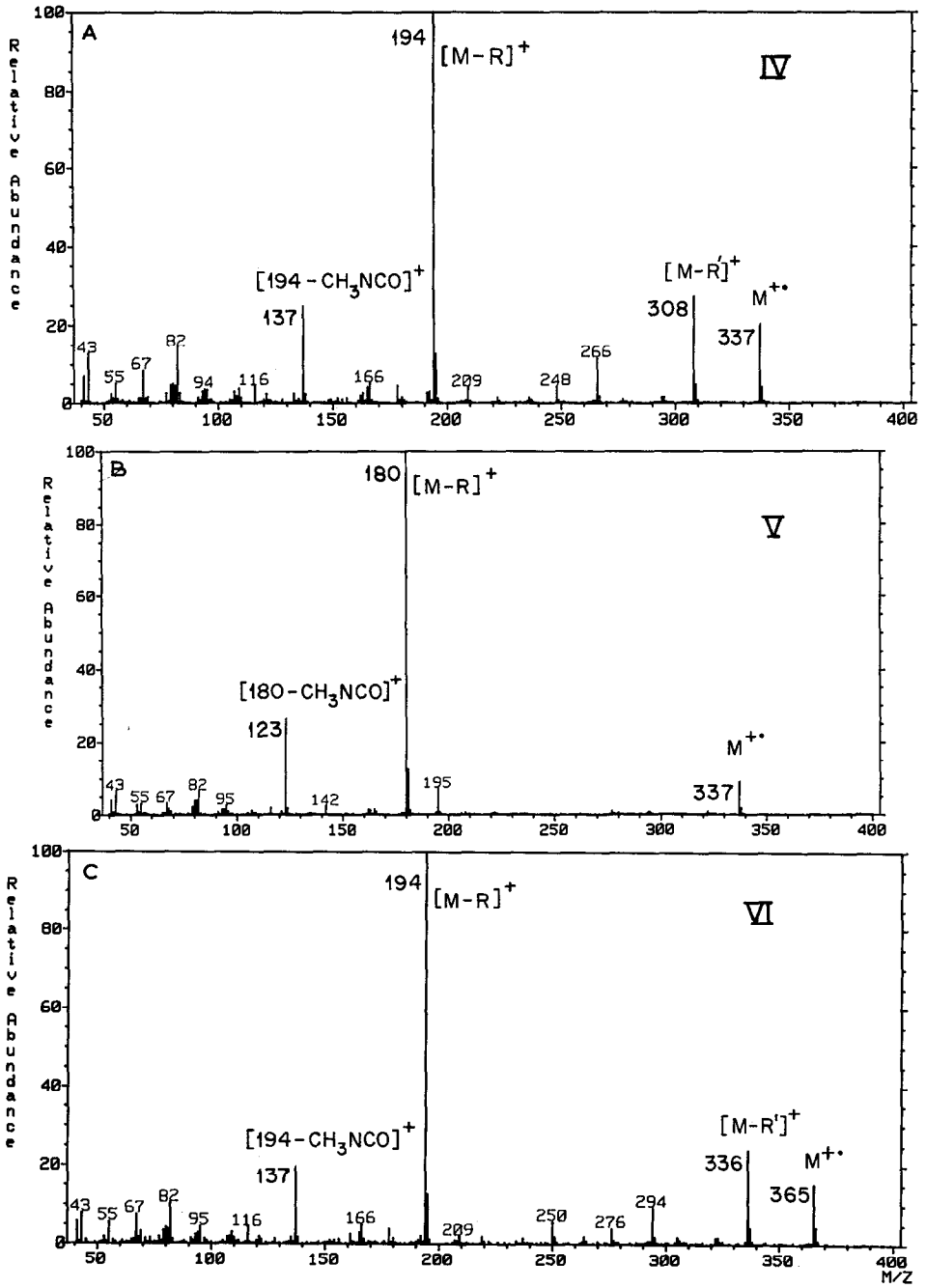

Fig. 6. (A) Electron impact mass spectra of MTAD derivatives of (A) 8*E*,10*E*-dodecadien-1-yl acetate (IV); (B) 7*E*,9*Z*-dodecadien-1-yl acetate (V); and (C) 9*Z*,11*E*-tetradecadien-1-yl acetate. (VI).

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