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Journal of Chromatography A, 803 (1998) 203–209

JOURNAL OF CHROMATOGRAPHY A

Gas-phase reagents for carbon–carbon double bond location: new applications
II. Use of vinyl methyl ether chemical ionization mass spectrometry to characterize C_{23} to C_{33} insect cuticular alkenes

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Received 28 July 1997; received in revised form 22 December 1997; accepted 22 December 1997

Abstract

The vinyl methyl ether (VME) reactivity towards alkenes R_1–CH=CH–R_2 in the gas phase giving rise to substituted VME radical cations $[R_1–CH=CH–OCH_3]^{+\circ}$ and/or $[R_2–CH=CH–OCH_3]^{+\circ}$ is a well known and elegant approach for the localization of the carbon–carbon double bonds. However, and despite the improvements proposed in the past to increase its specificity, real applications have rarely (if ever) been reported. In this paper, we describe the applicability of GC–VME chemical ionization as a method directly performed on crude cuticular extracts of social insects (three ant species namely *Myrmica alaskensis*, *Formicoxenus quebecensis* and *Formica selysi* and the honey bee). Assignment of the double bond position is done without ambiguity for a number of Δ^8, Δ^9 and Δ^{10} long chain alkenes (C_{23} to C_{33}) on the basis of the obtained substituted VME radical cations and corresponding ions after loss of methanol. © 1998 Elsevier Science B.V.

Keywords: Detection, GC; Double bond location; Mass spectrometry; Insects; Alkenes; Vinyl methyl ether

1. Introduction

Long chain hydrocarbons are major components of insect cuticular lipids. These substances prevent desiccation and are important in chemical communication, particularly for species and caste recognition [1,2]. Moreover, they can be used as a chemotaxonomic tool [3,4]. Solvent extraction of whole-body insects generally reveals the existence of numerous constituents: *n*-alkanes, monomethylalkanes, dimethylalkanes and alkenes. The location of the carbon–carbon double bond in the latter compounds often represents the main difficulty in achieving identification due to the small amounts available in natural samples.

Usually, the double bond position is determined by GC–MS analysis after condensed-phase microchemistry such as ozonolysis [5], epoxidation [6,7], alkylthiolation [8,9] etc. performed on isolated fractions. As an alternative to avoid this time- and material-consuming methodology, a direct chemical ionization mass spectrometry (CI-MS) approach based on specific ion-molecule reactions has been developed using a variety of reagent gases (or ions) including vinyl ethers [10–12], metal cations [13] and nitric oxide [14,15]. Some of these may present interesting reactivities towards model compounds but real structure elucidation work is rarely achieved.

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0021-9673/98/$19.00
PII S0021-9673(97)01285-5

However, in the case of monofunctional olefins [15] as well as for bifunctional ones (i.e. monounsaturated aldehydes, alcohols or acetates) [16], we have shown that NO^+-CI-MS (NO^+ being produced from nitric oxide) may be used with success for practical applications [17] despite a strong dependence on experimental factors. In fact, low sample pressure [15,16] and source temperature [18] are essential to provide characteristic acylium ion(s) on which the double bond assignment has to be based.

Although the application field of the NO^+-CI-MS method is still growing [19], some limitations may appear for compounds yielding insufficient or even ineffective production of the diagnostic acylium ions (effect of size and/or competition with other functions). The aim of this paper is to present an efficient alternative by using the vinyl methyl ether (VME) reagent [10–12] with which no real (i.e. biological, industrial, etc.) application was reported yet to our knowledge. The method appears particularly useful for the structure assignment of the very long chain alkenes of the type R_1–CH=CH–R_2 (R_1 and R_2 being alkyls) found as components of the cuticular lipids of three ant species and of the honey bee.

2. Experimental

2.1. GC–MS analysis

The VME-CI spectra were recorded on a GC–MS R10-10 Nermag (Quad Service, Poissy, France) quadrupole instrument under the following source conditions: temperature, 120°C; filament current, 100 μA; electron energy, 95 eV. VME (Fluka, Switzerland) and the O–C^2H_3 labelled reagent (98% purity, Cambridge Isotope Labs., Woburn, MA, USA) were used at a $5 \cdot 10^{-5}$ Torr pressure measured in the source housing (1 Torr=133.322 Pa). Under these conditions, the $VME^{+\circ}$ and $[VME+H]^+$ ions (m/z 58 and 59, respectively) were produced in approximately equal abundances. Additionally, using this plasma the relative intensities of the diagnostic ions obtained after reaction with alkene substrates were found to be optimal. Due to the presence of a variety of ions in the reagent plasma besides the main ones already cited, the VME-CI spectra were recorded in the 130–600 u mass range (1.1 scan/s). The chromatogram showing the electron impact ionization (EI) response in comparison to the above conditions was obtained using a 180°C source temperature, 200 μA as filament current and 70 eV electron energy. Samples were introduced via a Delsi 200 gas chromatograph equipped with a 25 m×0.32 mm I.D. fused-silica capillary column CPSil-5CB (Chrompack, Netherlands) and a Ross injector heated at 280°C. The temperature program used to analyze the insect cuticular extracts was from 200°C to 320°C at 8°C/min with helium as carrier gas at 0.75 bar inlet pressure.

2.2. Materials

The synthetic alkenes used as model compounds were prepared by conventional methods [20]. The natural extracts were generally obtained by 5-min maceration of whole-body insects (10 to 50 individuals for ants and a single one for honey bee) using 1–2 ml of pentane as solvent. Extracts were filtered on glasswool, concentrated under a gentle stream of N_2 to ~50 μl and analyzed without further purification (1–5 μl injected). Three ant species *Myrmica alaskensis*, *Formicoxenus quebecensis*, and *Formica selysi* and honey bees were utilized to prepare cuticular extracts.

3. Results and discussion

3.1. VME (and O–C^2H_3 labelled VME)-CI spectra of synthetic alkenes

In the high mass region, the VME-CI spectra of long chain alkenes (Z7: C_{23} and Z9: C_{23} were taken as model compounds) exhibit the adducts $[M+HVME]^+$, $[(M+CH_3CO]^+$, $[(M+VME)-CH_3OH]^{+\circ}$ and also the $[M-H_2]^{+\circ}$ ion as molecular species. The origin of these ions has been demonstrated elsewhere [21]. Besides this, the distinction between isomeric forms is made possible by two couples of 32 u distant ions whose m/z values are clearly related to the position of the double bond in the initial alkenes. As an example, ions of moderate relative abundances at m/z 170 and 138 and m/z 240 and 208 are observed for Z9: C_{23}. These diagnostic

ions are presumably the $[R_1–CH=CH–OCH_3]^{+\circ}$ (named $A^{+\circ}$, e.g. m/z 170 in this case) and $[R_2–CH=CH–OCH_3]^{+\circ}$ (named $B^{+\circ}$, e.g. m/z 240) and resulting ions after loss of MeOH (e.g. m/z 138 and 208, respectively) formed via an initial [2+2] cycloaddition reaction as was reported by Ferrer-Correia et al. [10,11]. The spectrum of Z9: C_{23} obtained with the $O–C^2H_3$-labelled VME is in agreement with this hypothesis since only the upper-mass ion in both couples, i.e. ions at m/z 170 and 240, are modified (+3 u shift) by the use of the deuterated reagent gas (Fig. 1). The resulting ions m/z 173 and 243 are thus of $[R–CH=CHOC^2H_3]^{+\circ}$ type whereas the unshifted ions correspond to the same ions after elimination of a C^2H_3OH molecule.

The limit of detection of the method was investigated. A 50-ng amount of a standard alkene appears sufficient to obtain a complete spectrum (scanning method) with diagnostic ions and major molecular species of significant relative intensities (signal-to-noise ratios measured through reconstructed single ion chromatograms were found as follows for Z9: C_{23} : S/N=8 for ion m/z 320 representing $[M–H_2]^{+\circ}$ and S/N=2 for ion m/z 170 (e.g. $A^{+\circ}$)). However, depending on their chromatographic complexity it is probable that the natural extracts may

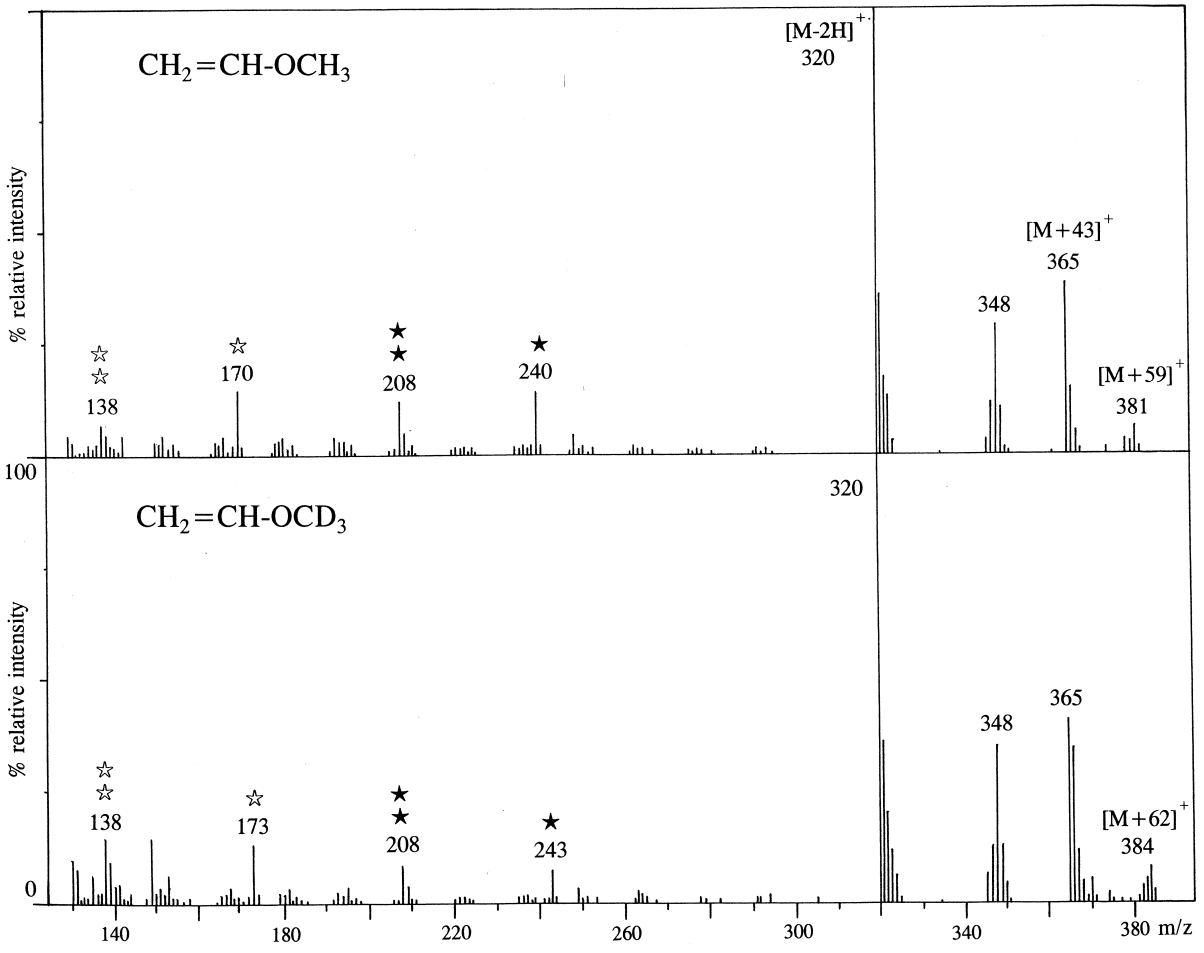

Fig. 1. Spectra of Z9: C_{23} synthetic alkene obtained under VME or $O–C^2H_3$-labelled GC–VME-CI-MS conditions. Open asterisk: $A^{+\circ}$; filled asterisk: $B^{+\circ}$; double asterisk: corresponding ions after loss of MeOH.

provide further limitation especially when isomeric mixtures are expected.

3.2. VME-CI spectra of natural long chain alkenes

3.2.1. Formicoxenus quebecensis and Myrmica alaskensis ant extracts

The integration of *Formicoxenus quebecensis* as parasitic ant in a host ant society (*Myrmica alaskensis*) was recently investigated [22] on a chemical basis by GC and GC–EI-MS analysis. The cuticular extracts from the two species being compared led to the observation of similar hydrocarbon (linear, methyl- or dimethyl-branched alkanes, linear monoalkenes) profiles.

The alkenes which were present in relatively large proportions were analysed in *Formicoxenus quebecensis* and *Myrmica alaskensis* by GC–VME-CI-MS to complete their characterization. They were found identical for both species and were all identified (Fig. 2) as homologues of the Z9: C_{23} standard. Indeed, their spectra displayed in common the same group of ions at m/z 170/138 stemming from the shortest alkyl side. These values corresponding to an ion of $[CH_3–(CH_2)_7–CH=CH–OCH_3]^{+\circ}$ structure and its fragment formed by elimination of CH_3OH were thus specific of the Δ^9 position of the unsaturation. The complementary ions observed at m/z 268/236, 296/264 and 324/292 respectively depended on the chain length. From both types of ions, the structures could be completely assigned as corresponding to the following alkenes: Δ^9: C_{25}, Δ^9: C_{27} and Δ^9: C_{29}. Their molecular masses could be confirmed from the main molecular species, e.g. $[(M+CH_3CO]^+$ and $[M-H_2]^{+\circ}$.

The observation of the same alkenes along with a variety of alkanes in both ant species is a strong argument for chemical mimicry in this host–parasite couple.

3.2.2. Formica selysi ant extract

Direct VME-CI analysis was also performed on the cuticular extract of another ant species *Formica selysi* which led us to confirm the characterization of numerous alkenes previously identified after alkylthiolation [23]. Examination of the spectra on the same basis as that discussed above (e.g. considering the $A^{+\circ}/B^{+\circ}$ diagnostic ions and the corresponding ions after loss of methanol, see Table 1) indicated that the unsaturation might occupy one of the three main following positions: Δ_8, Δ_9 and Δ_{10} (Table 1). The Δ_8 position was observed for two compounds of C_{29} and C_{31} chain length respectively. These components were identified by the presence of characteristic ions at m/z 156 ($A^{+\circ}$) and ions at m/z 338/306 and 366/334 respectively representing in each case $B^{+\circ}$ and $[B-CH_3OH]^{+\circ}$. The main Δ_9 alkenes were the same as those already mentioned e.g. Δ_9: $C_{25,}$ C_{27} and C_{29}. Ions at m/z 184/152 (specific for the Δ_{10} position) and at 338/306 and 366/334 unambiguously assigned the last alkenes as Δ_{10}: C_{31} and C_{33} respectively.

Under our conditions, this alkene containing extract was examined at a ~50-ng minimum GC injected dose for each unsaturated component. In contrast to the alkylthiolation method, the present approach is advantageous since it avoids the fractionation and/or purification of the extract which is necessary before performing the liquid phase derivatization. It thus prevents inherent material and time losses.

3.2.3. Honey bee (Apis mellifera) extract

The cuticular hydrocarbon composition was studied in the same way in the honey bee (extract of a single individual). Fig. 3 illustrates the total ion chromatogram obtained under the VME-CI conditions. The corresponding GC–EI-MS chromatogram (using an ~5-fold lower injected dose) is shown for comparison. The two chromatograms are very similar except for a variation in the alkene–alkane ratios indicating some difference in the responses of both types of molecules in function of the ionization method. The alkenes were Δ_8: C_{29} and C_{31}, Δ_9: C_{25} and C_{27} and Δ_{10}: C_{31} and C_{33}. Their identification was achieved on the basis of the same criteria as in the above study in particular using the same diagnostic ions to distinguish between the isomeric forms (Table 1). Although the alkene composition found for the honey bee cuticle is quite close to that of *Formica selysi*, the corresponding chromatographic profiles differ significantly: the Δ_8: C_{29}, Δ_9: C_{27} and Δ_9: C_{29} alkenes are major components in the latter whereas the honey bee alkenic profile is dominated by Δ_8: C_{31}, Δ_{10}: C_{31} and C_{33}.

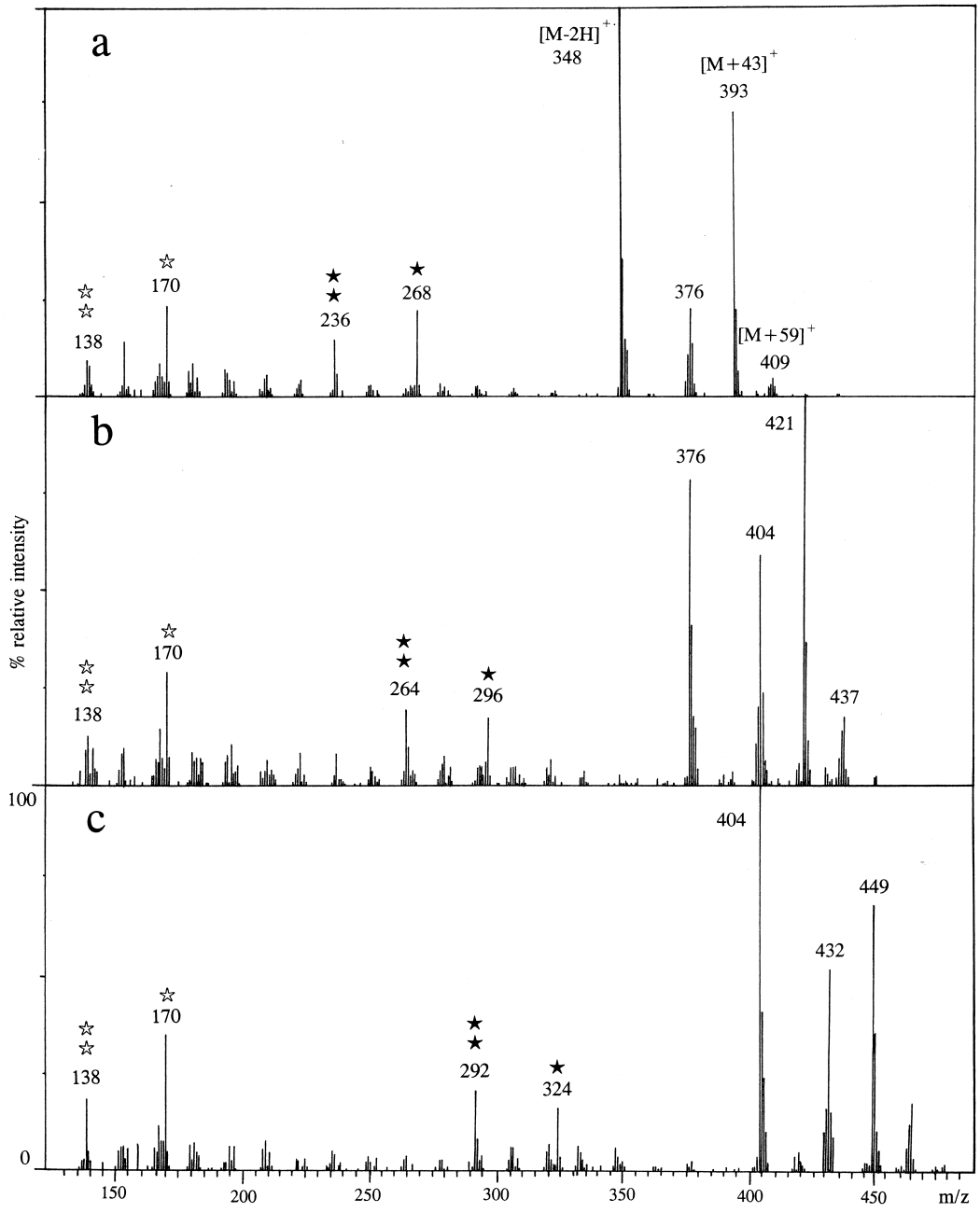

Fig. 2. Spectra of Δ_9: C_{25} (a), C_{27} (b) and C_{29} (c) alkenes from the cuticular extract of the ant *Myrmica alaskensis* obtained under GC–VME-CI-MS conditions. Legend as for Fig. 1.

4. Conclusion

Although very well known, the method of Ferrer-Correia et al. [10,11] based on the reactivity of the VME reagent towards carbon–carbon double bonds has been rarely utilized for applications. This can be

Table 1
VME–CI diagnostic ions of synthetic and natural alkenes from *F. selysi* (bold) and the honey bee

	A	A−MeOH	B	B−MeOH	M−H_2	M+43	M+59
Z7: C_{23}	142 (27)		268 (9)	236 (13)	320 (100)	365 (54)	381 (7)
Z9: C_{23}	170 (25)	138 (4)	240 (12)	208 (11)	320 (100)	365 (54)	381 (7)
9: C_{23}	**170 (16)**	**138 (10)**	**240 (14)**	**208 (17)**	**320 (100)**	**365 (55)**	**381 (5)**
9: C_{25}	170 (19)	138 (13)	268 (9)	236 (17)	348 (100)	393 (73)	409 (13)
	(16)	**(11)**	**(14)**	**(21)**	**(100)**	**(62)**	**(4)**
9: C_{26}	**170 (17)**	**138 (11)**	**282 (17)**	**250 (22)**	**362 (100)**	**407 (64)**	**423 (5)**
9: C_{27}	170 (15)	138 (12)	296 (7)	264 (17)	376 (100)	421 (67)	437 (5)
	(17)	**(11)**	**(15)**	**(24)**	**(100)**	**(67)**	**(7)**
9: C_{28}	**170 (18)**	**138 (11)**	**310 (12)**	**278 (21)**	**390 (100)**	**435 (62)**	**451 (8)**
8: C_{29}	156 (26)		338 (10)	306 (23)	404 (100)	449 (58)	465 (10)
	(28)		**(14)**	**(5)**	**(45)**	**(100)**	**(10)**
9: C_{29}	**170 (16)**	**138 (8)**	**324 (13)**	**292 (14)**	**404 (83)**	**449 (100)**	**465 (12)**
8: C_{31}	156 (42)		366 (11)	334 (27)	432 (100)	477 (72)	493 (10)
	(23)		**(9)**	**(16)**	**(49)**	**(100)**	**(14)**
10: C_{31}	184 (46)	152 (24)	338 (17)	306 (48)	432 (100)	477 (72)	493 (10)
	(27)	**152 (9)**	**(16)**	**(14)**	**(98)**	**(100)**	**(14)**
10: C_{33}	184 (17)	152 (11)	366 (6)	334 (17)	460 (100)	505 (49)	521 (7)
	(17)	**152 (7)**	**(5)**	**(10)**	**(100)**	**(51)**	**(7)**

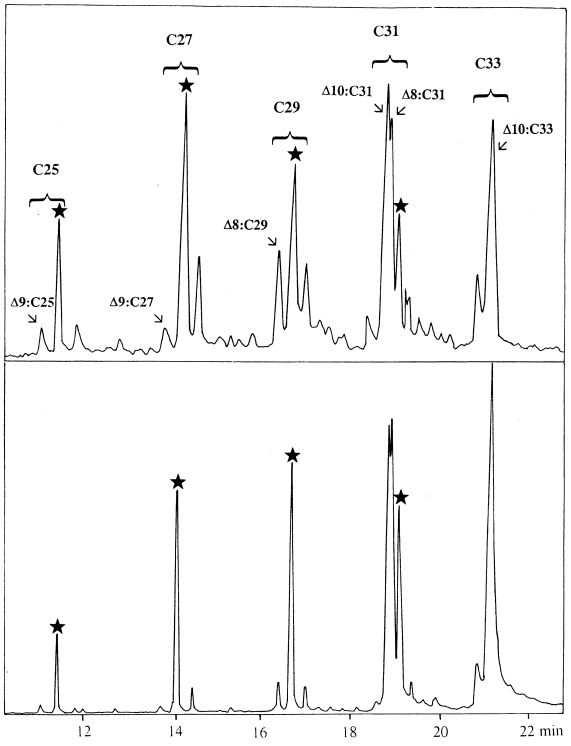

Fig. 3. Total ion chromatograms obtained from the cuticular extract of honey bee under VME-CI (above) and EI (below) conditions. The identified alkenes are mentioned and corresponding linear alkanes are labelled with an asterisk.

explained by the complex plasma being formed under the high pressure source conditions necessary for CI which provides relatively high mass and/or undesirable ions in the background. Moreover, the proposed improvements (mixture of N_2–CS_2–VME) [24] to reduce the complexity of the plasma and further to increase the selectivity of the reaction were not followed either. In our study, however, we have shown that the VME-CI method may be quite satisfactory to assign the double bond position in natural long chain alkenes ranging from C_{23} to C_{33}. In such cases, the m/z values of the diagnostic ions may be high enough to overcome the problem of the plasma background, especially when $\Delta \geq 7$. For lower Δs, the assignment should be based on the $B^{+\circ}/[B-CH_3OH]^{+\circ}$ ions – that contain the longest alkyl chain – only.

Acknowledgements

The authors are grateful to A. Lenoir (CNRS, Villetaneuse, France) and G. Arnold (INRA, Bures-sur-Yvette, France) for providing respectively the ant and honeybee extracts.

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