JOURNAL OF MASS SPECTROMETRY, VOL. 32, 723–727 (1997)

Gas-phase Reagents for Carbon–Carbon Double Bond Location: New Applications

Part I—Nitric Oxide Chemical Ionization Mass Spectrometry of 1-*O*-Alkenylglycerols from the Deep-sea Shark *Centrophorus Squamosus* Liver Oil

Nicole Sellier,[1] Catherine Bordier,[1] Lucien Kerhoas[2] and Jacques Einhorn[2]*
[1] Laboratoire de Spectrométrie de Masse, ENSCP–CNRS, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France
[2] Unité de Phytopharmacie et Médiateurs Chimiques, INRA, Route de St Cyr, 78026 Versailles Cedex, France

Nitric oxide chemical ionization (CI) mass spectrometry (MS) has proved to be a very efficient tool for locating the carbon–carbon double bond in monoalkenylglycerols of the $HOCH_2CHOHCH_2O(CH_2)_nCH{=}CH(CH_2)_mCH_3$ type. Such compounds, e.g. containing a $\omega-7$ C_{16}:1, $\omega-8$ C_{17}:1 (minor) and $\omega-9$ C_{18}:1 alkenyl chain, were extracted from the liver oil of the deep-sea shark *Centrophorus squamosus* and found among the most characteristic substances of the species. Double bond location was unambiguously deduced from the abundant $CH_3(CH_2)_mCO^+$ acylium ion being produced when examining the permethylated compounds under gas chromatographic (GC) nitric oxide CIMS conditions. This ion probably results, as suggested by GC/MS/MS studies, from mechanisms similar to those previously reported for mono- or bifunctional alkenes. © **1997 by John Wiley & Sons, Ltd.**

J. Mass Spectrom. **32**, 723–727 (1997)
No. of Figures: 5 No. of Tables: 0 No. of Refs: 23

KEYWORDS: double bond location; alkenylglycerol; nitric oxide; chemical ionization mass spectrometry; deep-sea shark

INTRODUCTION

Occurring as major constituents of the liver oil in various species of deep-sea sharks,[1] glycerol ethers may represent an interesting source for industrial applications. These compounds are known to exhibit bacteriostatic and anti-inflammatory properties,[2] as well as hemopoietic and neuromuscular effects.[3,4] They are also believed to protect against radiation damage[5] and to possess antitumor properties in their phospholipid form.[6] For these reasons, we recently investigated the lipidic composition of the deep-sea shark *Centrophorus squamosus*. After characterization of the fatty acids (countercurrent chromatography being used to isolate the unsaturated fatty acids,[7] the glycerol ether content was examined.[8] The alkenylglycerols were specifically separated (from alkylglycerols) for identification.

Although several ionization methods and approaches have been proposed for the analysis of glycerides,[9,10] the literature on glycerol ethers is relatively limited.[11,12] Branch points, for instance, can be determined in 1-*O*-alkylglycerols through, e.g., nicotinylidene derivatives.[12] However, there is no specific method for the rapid characterization of the unsaturation in alkenylglycerol structures occurring in natural mixtures. Our continuing interest in gas-phase ion–molecule reactions to localize functional groups[13] thus led us to investigate the possible application of gas chromatography/nitric oxide chemical ionization mass spectrometry (GC/NO-CIMS).

After initial studies by Hunt and Harvey[14] on the electrophilic reactivity of NO^+ towards alkenes, Budzikiewicz and Busker[15] made the first observations as to the possible use of NO-CIMS for locating double bonds. Their proposal was based on NO-containing ions of low abundance stemming from the $[M + NO]^+$ adduct ion. In 1986, we reported[16] for long-chain alkenols (and their acetates) new and highly abundant diagnostic ions of the acylium type. The occurrence of such ions was subsequently generalized to other bifunctional alkenes and to alkenes[17,18] and shown to be optimally produced at low ion-source temperatures,[17] low pressures (e.g. small amounts introduced via GC) of substrate and high NO pressures.[18,19] According to these observations and other studies, we suggested[18,19] two possible mechanisms, involving (i) the decomposition of an excited adduct ion through reaction with $NO^{\cdot}$:

$$[R_1CH{=}CHR_2 + NO]^{+}* + NO^{\cdot} \rightarrow R_1CO^+(A) \text{ and/or } R_2CO^+(B)$$

(ii) a surface-catalysed reaction with neutral $NO^{\cdot}$ to give aldehydes:

$$R_1CH{=}CHR_2 \xrightarrow{NO^{\cdot}} R_1CHO \text{ and/or } R_2CHO$$

which thereafter undergo hydride abstraction with NO^+ to yield acylium ions.

* *Correspondence to*: J. Einhorn, Unité de Phytopharmacie et Médiateurs Chimiques, INRA, Route de St Cyr, 78026 Versailles Cedex, France.

CCC 1076–5174/97/070723–05 $17.50

Received 23 October 1996
Accepted 27 March 1997

Based on diagnostic acylium ions, GC/NO-CIMS is routinely used in our group for the structural elucidation (i.e. CC double location) of trace compounds from biological sources.[20,21]

EXPERIMENTAL

Electron impact (EI) ionization and NO-CI mass spectra were recorded using an R-10-10 quadrupolar GC/MS (or R-30-10, e.g. MS/MS) system (Nermag, Argenteuil, France) with the following source conditions: temperature, 120 °C; filament current, 50 μA; and electron energy, 70 eV (EI) or 90 eV (CI). Nitric oxide (99.9%) from Air Liquide (Paris, France) as reagent gas was used at a 0.1 Torr pressure ($\sim 10^{-4}$ Torr in the source housing) (1 Torr = 133.3 Pa). GC/NO-CIMS/MS was performed with the R-30-10 instrument at 30 eV collision energy and using Ar at 4×10^{-2} Torr in the collision cell.

Samples (optimally 50–200 ng of each component) were introduced with a Ros injector via a 25 m × 0.23 mm i.d. fused-silica capillary column coated with CP-Sil 5CB (Chrompack, Middelburg, The Netherlands). The carrier gas was helium. The oven temperature was programmed from 190 to 285 °C at 10 °C min^{-1}.

Shark liver oil was obtained from IS-France (Lorient, France). It was first saponified then esterified with BF_3–MeOH (cf. methyl ester derivatives of the fatty acid content). The glycerol ether fraction was obtained after silica gel chromatography and the monoalkenylglycerols were specifically separated using urea complexation.[8] These compounds were finally methylated (CH_3I–NaH–DMSO, 30 min). After the usual work-up, the mixture was analyzed without further purification by GC/NO-CIMS.

RESULTS AND DISCUSSION

EI spectra of the monoalkenyldimethylglycerols

The isolated fraction of permethylated monoalkenylglycerols (see above) was first examined by GC/EIMS. In this analysis, two main compounds (**1** and **3**) and a very minor one (**2**) were observed (named according to their elution order). Comparison of the data led to the following: (i) the presence of the molecular ion $M^{+\cdot}$ and subsequent ions $[M - CH_3OH]^{+\cdot}$, $[M - CH_3OCH_2^{\cdot}]^+$ and $[M - CH_3OCH_2^{\cdot} - CH_3OH]^+$ of low relative intensities, (ii) ions at *m/z* 121, presumably of the protonated dimethylglycerol type and a complementary ion $[M - 120]^{+\cdot}$ (iii) even-electron ions of low *m/z*, e.g. 103 and 89 resulting from the *m/z* 121 ion by loss of water or methanol and other oxygenated ions (*m/z* 59 and 45). Finally, the spectra also exhibited alkenyl ions (*m/z* 55 as base peak) which stem from the aliphatic chain. All these data are in favor of monoalkenylglycerol structures with M_r 342, 356 and 370, respectively, thus corresponding to $C_{16}:1$, $C_{17}:1$ (minor) and $C_{18}:1$ alkenyldimethylglycerols (Fig. 1, spectrum of $C_{18}:1$ as an example). The observation of *m/z* 89 and $[M - CH_3OCH_2^{\cdot}]^+$ ions allows the assignment of a 1-*O*-alkenyl substitution in the three compounds. However, there is no indication as to the double bond position in the chain.

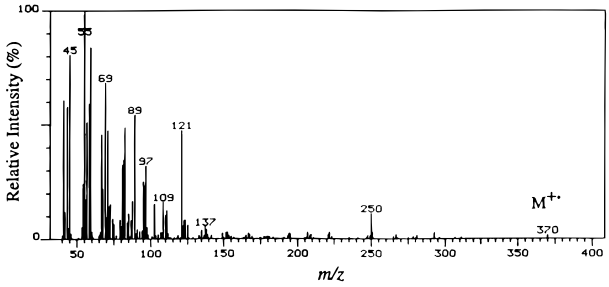


Figure 1. EI mass spectrum of (permethylated) 1-*O*-alkenylglycerol **3** from *Centrophorus squamosus* liver oil.

NO-CI spectra of the monoalkenyldimethylglycerols

The NO-CI spectra (Fig. 2) obtained from the three alkenyl(permethylated)glycerols investigated exhibited a number of molecular ion species. These ions reflect the various reactivities which generally arise from the use of NO as reagent: (i) adduct $[M + NO]^+$ and minor $[M + NO - H_2O]^+$ ions resulting from electrophilic condensation, (ii) $M^{+\cdot}$ and $[M - 120]^{+\cdot}$ ions generated by charge exchange and (iii) $[M - H]^+$ formed by hydride abstraction. The last ion is accompanied by fragment

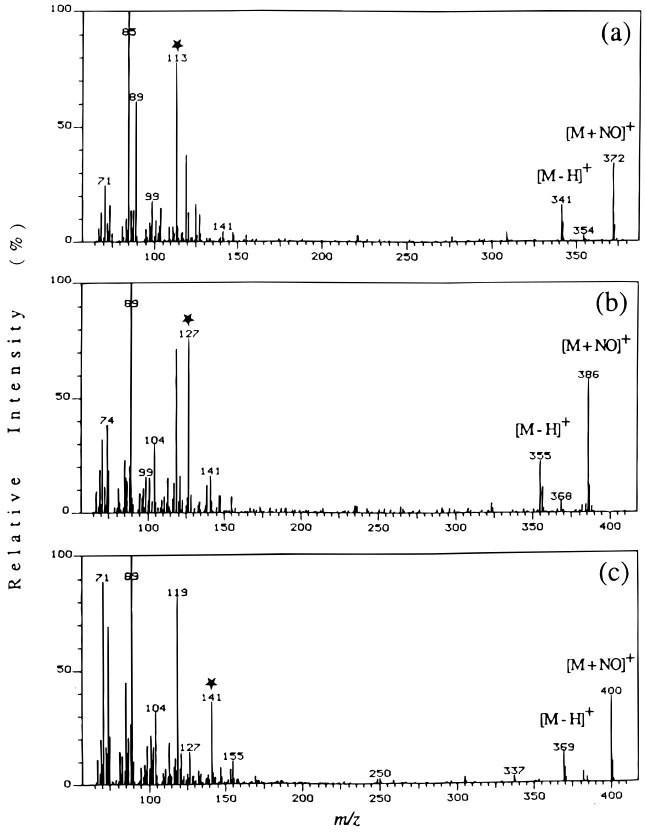
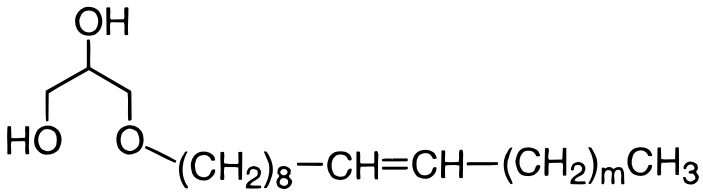


Figure 2. NO-CI mass spectra of (permethylated) (a) $\omega-7$ $C_{16}:1$, (b) $\omega-8$ $C_{17}:1$ and (c) $\omega-9$ $C_{18}:1$ 1-*O*-alkenylglycerols **1–3** from *Centrophorus squamosus* liver oil. Asterisks indicate acylium diagnostic ion.



1 (m = 5)
2 (m = 6)
3 (m = 7)

Figure 3. Structures of the 1-*O*-alkenylglycerols **1**–**3** from *Centrophorus squamosus* liver oil.

ions of low abundances arising from the loss of one (or two) methanol molecule(s). All these ions confirm the molecular masses and some of the main characteristics of the compounds investigated.

In the low-mass region, ions at *m/z* 121, 119, 103 and 89 (*m/z* 119 and 89 being of high relative abundance) are present for the three compounds and are similar to or identical (e.g. *m/z* 121, 103 and 89) with ions appearing in the EI spectra and thus characterize the common glycerol-type skeleton (see the collision activated dissociation (CAD) studies].

However, the most interesting feature is the occurrence of an abundant odd-mass ion whose *m/z* value is related to the position of the double bond in the alkenyl chain. According to these values, e.g. *m/z* 113, 127 and 141 for (permethylated) **1**, **2** and **3**, respectively,

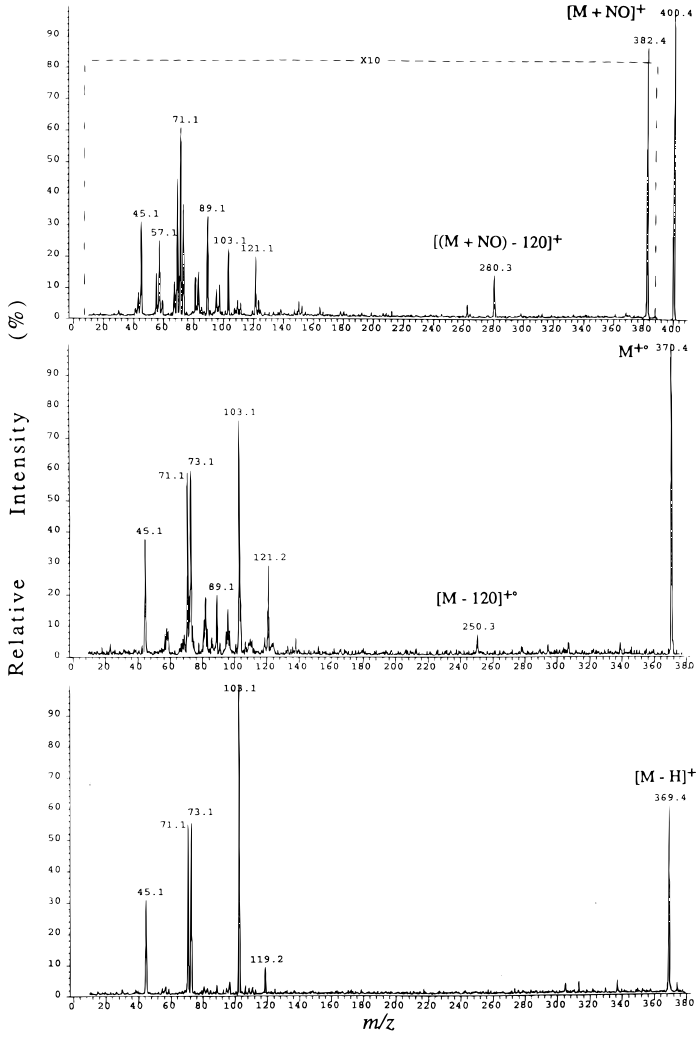


Figure 4. CAD fragment ion spectra of the (a) $[M + NO]^+$, (b) $M^{+\cdot}$ and (c) $[M - H]^+$ ions obtained from $\omega - 9$ C_{18}:1 1-*O*-alkenylglycerol **3** (GC/NO-CIMS/MS conditions).

an acylium ion structure generated from the non-functionalized end of the alkenyl chain in each compound can be immediately suspected. The nature of such an RCO^+ diagnostic ion is further evidenced by the presence of R^+ ions resulting from the loss of CO in the spectra. In the case of **1**, R^+ appears as the base peak at *m/z* 85. These data would then assign the unsaturation at the $\omega - 7$, $\omega - 8$ and $\omega - 9$ positions (or same Δ^9 position relative to the 1-oxygen function) in **1** (1-*O*-hexadec-9-enylglycerol), **2** (1-*O*-heptadec-9-enylglycerol) and **3** (1-*O*-octadec-9-enylglycerol), respectively (Fig. 3).

The above conclusions have been verified[22] and found identical by applying a conventional procedure of condensed-phase double bond derivatization on (permethylated) **1** and **3** (the most abundant). The dimethoxy derivatives obtained[23] gave rise under EI to ions at *m/z* 275 and 129 (**1**) and at *m/z* 275 and 157 (**3**), as expected.

CAD studies

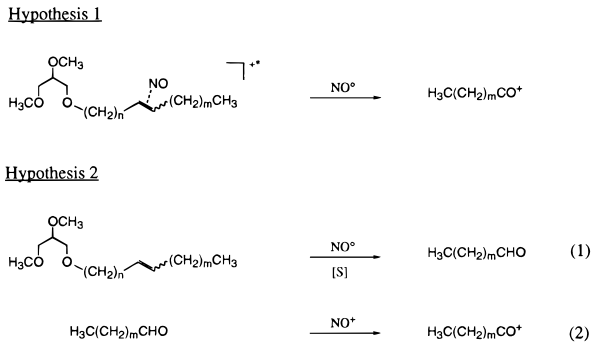
Low-energy CAD studies were undertaken (cf. compound **3**) to determine the origin of the acylium diagnostic ion and other characteristic ions.

The CAD fragment ion spectra (Fig. 4) from the $M^{+\cdot}$ and $[M + NO]^+$ ions gave rise to similar spectra showing, in addition to the possible initial loss of H_2O in the latter, two main processes of decomposition. The first leads, after initial charge exchange ($M^{+\cdot}$) or adduct-ion formation ($[M + NO]^+$), to the production of protonated 1,2-dimethylglycerol (*m/z* 121). The second yields a complementary ion of either $[M - 120]^{+\cdot}$ or $[(M + NO) - 120]^+$ structure. The other main fragment ions result from further dissociation of the *m/z* 121 ion, either by loss of water (*m/z* 103) or methanol (*m/z* 89) or by the elimination of two neutrals, e.g. $[121 - (H_2O + CH_3OH)]^+$ at *m/z* 71 or $[121 - (H_2O + CH_2O)]^+$ at *m/z* 73. Regarding $[M + NO]^+$ formation, these data strongly indicate that the initial electrophilic addition of NO^+ occurs on the double bond of the alkenylglycerol substrate.

The CAD fragment ion spectrum obtained from the $[M - H]^+$ ion is relatively simple (Fig. 4). The ion at *m/z* 103 (and subsequent fragments at *m/z* 71 and 73), also observed in the above CAD spectra, can be assumed in this case to result from an initial hydride abstraction at the first carbon of the ether chain and its elimination as a long-chain unsaturated aldehyde ($C_{17}H_{33}CHO$). The ion at *m/z* 119 might then be produced through the same initial $[M - H]^+$ ion after 1,2-H transfer followed by the loss of a long-chain diene ($C_{16}H_{31}CH{=}CH_2$).

Origin of the acylium diagnostic ion

The acylium diagnostic ion at *m/z* 141 does not occur in any of the above CAD spectra obtained from compound **3**, which suggests, for its origin, similar mechanisms (Scheme 1) to those reported for aliphatic or functionalized alkenes.[18,19] However, although both proposals are consistent with the experimental parameter effects (particularly NO pressure and amount of substrate),[18,19] hypothesis (2) should be preferred owing to the broadening and slight delay observed for the acylium ion *m/z* 141 GC/MS peak *vs.* any of those of the molecular ion species. Isomerization may occur in some extent during this process since homologous ions of lower abundances are also produced (ions shifted by ± 14 u). It is probable, however, that most ions observed in the low-mass region are alkyl fragments formed after the loss of CO followed eventually by further elimination of neutral alkenes. For instance, in the case of compound **1**, it was confirmed by CAD experiments that the ion at *m/z* 85 ($C_6H_{13}^+$ according to the fragment ion spectrum pattern) was correlated with the ion at *m/z* 113 (identified as $C_6H_{13}CO^+$ by its CAD spectrum exhibiting ions at *m/z* 85 (loss of CO) and 43 (elimination of $[CO + C_3H_6]$)). In the conventional spectrum (Fig. 2), the ion at *m/z* 85 was highly abundant as compared with the corresponding fragments obtained from higher acylium homologues (cf. compounds **2** and **3**) as a result probably of its limited subsequent fragmentation (size effect).



Scheme 1. Possible origin of the acylium diagnostic ion assigning the double bond position (two hypotheses).

CONCLUSION

The suitability of $NO^+/NO^{\cdot}$ as gas-phase reactant for the localization of the double bond of 1-*O*-alkenylglycerols of natural origin (i.e. liver oil of the deep-sea shark *Centrophorus squamosus*) has been demonstrated. Under these conditions, an acylium diagnostic ion was generated from the non-functionalized end of the alkenyl chain. The occurrence of a single acylium ion (no complementary ion stemming from the oxygen function side) was also commonly observed in most bifunctional alkenes.[17,19] Using this method, $C_{16}:1$, $C_{17}:1$ and $C_{18}:1$ compounds present in the same fraction were characterized with the unsaturation at the $\omega - 7$, $\omega - 8$ and $\omega - 9$ positions, respectively. Owing to the presence of the ± 14 u homologous ions, these assignments could be done, however, only after careful GC separation (cf. GC/MS conditions). In this case, each of the major components was proved to contain a single isomeric form using a conventional procedure of double bond derivatization. Although the geometry was not determined (accessible rather by GC/Fourier transform infrared spectrometry) in any of these components, the $C_{16}:1$ and $C_{18}:1$ structures were rarely found and the odd-carbon alkenylglycerol is original.

REFERENCES

1. M. J. Bakes and P. D. Nichols, *Comp. Biochem. Physiol.* **110B**, 267 (1995).
2. R. G. Burford and C. W. Gowdey, *Arch Int. Pharmacodyn.* **173**, 56 (1968).
3. B. Boeryd, T. Nilsson, L. Lindholm, L. Lange, B. Hallgren and G. Ställberg, *Eur. J. Immunol.* **8**, 678 (1978).
4. S. Brohult and A. Brohult, *Swed. Pat.* 0333678 (1989).
5. A. Brohult, J. Brohult, S. Brohult and I. Joelsson, *Acta Obstet. Gynecol. Scand.* **65**, 779 (1986).
6. D. E. Vance and J. E. Vance, *Biochemistry of Lipids and Membranes*. Benjamin/Cummings, Menlo Park, CA (1985).
7. C. G. Bordier, N. Sellier, A. P. Foucault and F. Le Goffic, *Chromatographia* **39**, 329 (1994).
8. C. G. Bordier, N. Sellier, A. P. Foucault and F. Le Goffic, *Lipids* **31**, 521 (1996).
9. C. Evans, P. Traldi, M. Bambagiotti-Alberti, V. Giannellini, S. A. Coran and F. F. Vincieri, *Biol. Mass Spectrom.* **20**, 351 (1991), and references cited therein.
10. K. L. Duffin, J. D. Henion and J. J. Shieh, *Anal. Chem.* **63**, 1781 (1991).
11. D. J. Harvey, *Biol. Mass Spectrom.* **20**, 61 (1991).
12. D. J. Harvey, *Biol. Mass Spectrom.* **20**, 87 (1991).
13. J. Einhorn, in *Therapeutic Aspects and Analytical Methods in Cancer Research*, *Mass Spectrometry in Cancer Research* (Proceedings), edited by E. Constantin, p. 206/1 Amudes, Strasbourg (1994).
14. D. F. Hunt and T. M. Harvey, *Anal. Chem.* **47**, 2136 (1975).
15. H. Budzikiewicz and E. Busker, *Tetrahedron*, **36**, 255 (1980).
16. C. Malosse and J. Einhorn, *Adv. Mass Spectrom.* **10B**, 1369 (1986).
17. H. Budzikiewicz, B. Schneider, E. Busker, W. Boland and W. Francke, *Org. Mass Spectrom.* **22**, 458 (1987).
18. J. Einhorn and C. Malosse, *Org. Mass Spectrom.* **25**, 49 (1990).
19. C. Malosse and J. Einhorn, *Anal. Chem.* **62**, 287 (1990).
20. C. Descoins, C. Malosse, M. Renou, B. Lalanne-Cassou and J. Le Duchat d'Aubigny, *Experientia*, **46**, 536 (1990).
21. G. Febvay, G. Bonnot, C. Malosse and J. Einhorn, *Experientia*, **49**, 915 (1993).
22. C. G. Bordier, Doctoral Thesis, Université Paris VI, pp. 240–243 (1995).
23. W. G. Niehaus and R. Ryhage, *Anal. Chem.* **40**, 1840 (1968).