

Location of Deep-Sea Shark (*Centrophorus squamosus*) Glycerol Ether Lipid Double Bonds by Nitric Oxide Chemical Ionization Mass Spectrometry

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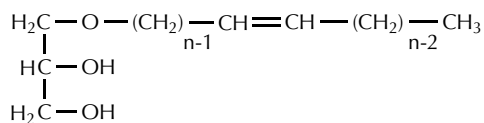
ABSTRACT: Chemical ionization mass spectrometry with nitric oxide as the reactant gas has proved to be an efficient tool for locating the double bond in monoalkenylglycerols of type HO-CH₂-CHOH-CH₂-O-(CH₂)_{n-1}-CH=CH-(CH₂)_{n-2}-CH₃. These monoalkenylglycerols were extracted from the liver oil of the shark *Centrophorus squamosus*, which lives at depths ranging from 1000 m and deeper. The presence of characteristic acylium ions R₁CO⁺ and/or R₂CO⁺ allowed location of the double-bond position.

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KEY WORDS: Characteristic acylium ions, chemical ionization with NO⁺, ether di-*O*-methylglycerols, gas chromatography/mass spectrometry, Glycerol ethers, hexadec-9-enylglycerol, liver oil of shark *Centrophorus squamosus*, location of double bond in monoalkenylglycerols, octadec-9-enylglycerol, selachyl alcohol.

Subsequent to studies by Hunt and Harvey (1), Einhorn (2–4), and Budzikiewicz (5,6) on the reaction of NO⁺ with olefins, chemical ionization nitric oxide mass spectrometry (CI/NO⁺/MS) was used for locating double bonds in aliphatic compounds. The purpose of this study was to examine the possible application of CI/NO⁺/MS for double-bond location in 1-*O*-monoalkenylglycerols isolated from the liver oil of the shark *Centrophorus squamosus*, which are glycerol ether lipids as shown in Scheme 1.

We recently reported (7) the characterization of fatty acid methyl esters from this liver oil by gas chromatography–mass spectrometry (GC–MS), and the purification of two of them,



SCHEME 1

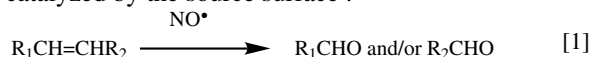
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namely docosahexaenoic acid (DHA or 22:6) and docosamonoenoic acid (DMA or 22:1), by countercurrent chromatography. We then directed our investigations to the unsaponifiable complementary fraction, more particularly to the glycerol ether fraction (8), because of its potential applications in industry.

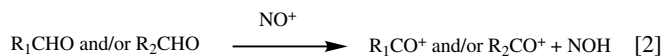
The unsaponifiable matter of this liver oil contains glycerol ethers in addition to squalene. These molecules exhibit bacteriostatic (9) and antiinflammatory properties (10) and hemopoietic effects (11,12); they are also believed to protect against radiation damage (13) and possess antitumor properties (14).

Electronic impact (EI) of pure NO in a conventional CI source produces NO⁺ in good yield with lesser amounts of the dimeric ion (NO)₂⁺. In general, three alternative reaction channels (15) are observed and give (M + NO)⁺, (M – H)⁺, and M⁺ ions.

Apparently two distinct processes may occur (3,4): Step 1: oxidative cleavage of the double bond and neutral-neutral reaction catalyzed by the source surface :



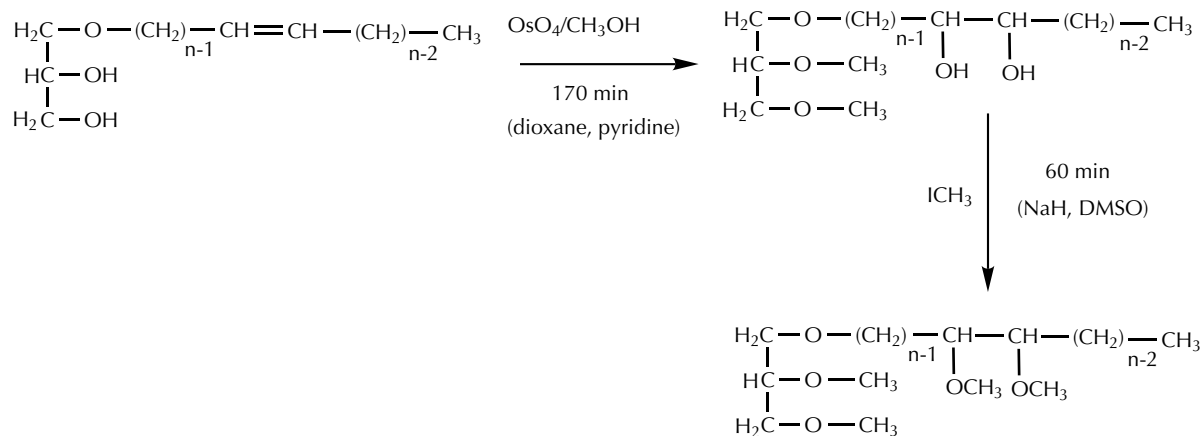
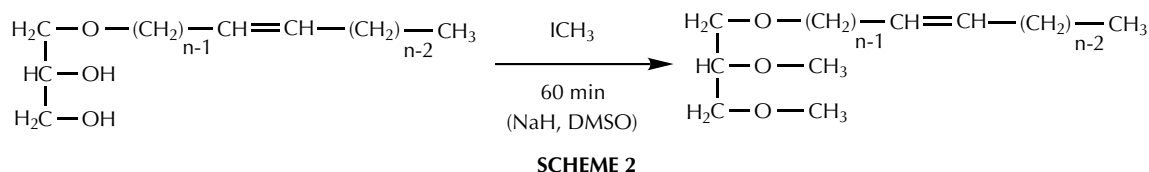
which may be followed by Step 2: hydride ion abstraction, classically observed with NO as reactant gas:



The ions R₁CO⁺ and/or R₂CO⁺ are acylium diagnostic ions. The most important prerequisites for the formation of these acylium ions are a low ion-source temperature (16) and a small amount of sample at the given NO pressure (3,4).

EXPERIMENTAL PROCEDURES

Shark liver oil was obtained from IS-France Company (Lorient, France). By a procedure already described (8), the oil was saponified and esterified with BF₃ in methanol; after fractionation of the derivatized oil by chromatography on silicic acid, monounsaturated glycerol ethers were separated, homologous *via urea* complexation, from the glycerol ether fraction.



The purified mixed 1-*O*-monoalkenylglycerols were then first methylated (17) to obtain the corresponding ether di-*O*-methylglycerols according to Scheme 2.

To obtain the 1-*O*-dimethoxymonoalkyl-di-*O*-methylglycerol derivatives (see below), the purified mixed 1-*O*-monoalkenylglycerols were secondly methoxylated by using a procedure already described by Hallgren *et al.* (18) (Scheme 3).

Samples were introduced with a Ros injector, *via* a 25 m × 0.23 mm capillary fused-silica column, coated with CP-Sil 5CB (Chrompack, The Netherlands). The carrier gas was helium with a flow velocity of 35.3 cm·s⁻¹. Oven temperature was programmed from 190 to 285°C at 10°C·min⁻¹.

The CI/NO⁺ mass spectra of ether di-*O*-methylglycerols and the EI mass spectra of 1-*O*-dimethoxymonoalkyl-di-*O*-methylglycerols were recorded with Nermag R30-10 GC-MS equipment (Delsi-Nermag, Argenteuil, France), under the following conditions: T_{source} 120°C, filament current 50 μA (CI) or 200 μA (EI), electron energy 90 eV (CI) or 70 eV (EI); NO (99.9% from Air Liquide, France) at 0.1 Torr pressure (or 10⁻⁴ Torr in the source housing).

RESULTS AND DISCUSSION

The chromatogram of the purified mixed ether di-*O*-methylglycerols is given in Figure 1. The CI/NO⁺ mass spectra (Fig.

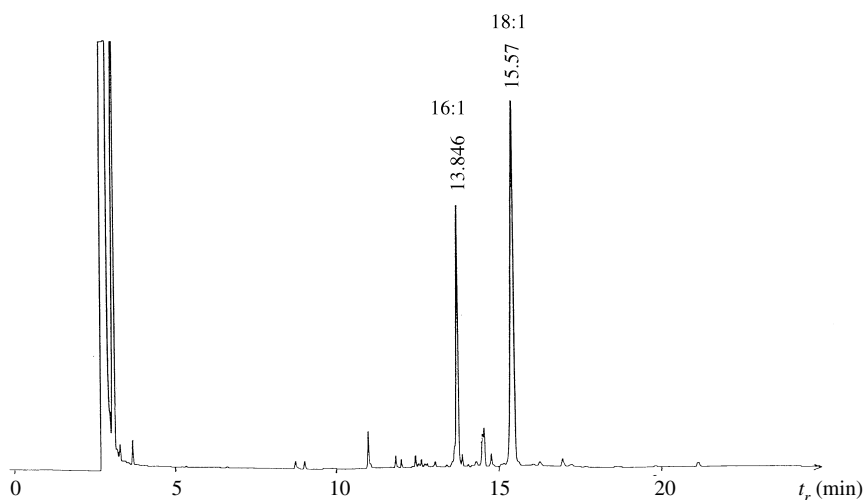


FIG. 1. Chromatogram of the purified mixed ether di-*O*-methylglycerols.

2A,B) obtained from the two 1-*O*-monoalkenyl-di-*O*-methylglycerols with retention times of 13.85 min and 15.57 min (see Fig. 1), respectively, exhibited ions at m/z 372 (A) and m/z 400 (B), corresponding to the $(M + NO)^+$ adduct ion, and m/z 341 (A) and m/z 369 (B), corresponding to the $(M - H)^+$ hydride ion abstraction (15).

More interesting was the presence in the lower mass region of abundant odd-mass ions whose m/z values were clearly related to the position of the double bond. In mass spectrum (A), we observed ions m/z 113 and m/z 85 (base peak); m/z 113 is the diagnostic acylium ion $CH_3-(CH_2)_5-C=O^+$, and m/z 85 is the ion $CH_3-(CH_2)_4-CH_2^+$, corresponds to the loss of CO from the acylium ion. The presence of acylium ion m/z 113 proved that the unsaturation of this glycerol ether was in the ω -7 position. In mass spectrum (B), we observed the ion m/z 141, which was the diagnostic acylium ion $CH_3-(CH_2)_7-C=O^+$, and the ion m/z 113 $CH_3-(CH_2)_6-CH_2^+$, which corresponded to the loss of CO from the acylium ion. These results confirmed that the unsaturation of this glycerol ether was in the ω -9 position.

The ions m/z 309 (A), m/z 277 (A), m/z 337 (B) ($M - H - CH_3OH$), and m/z 305 (B) ($M - H - 2CH_3OH$) arose, respectively, from the loss of CH_3OH once and twice from the hydride ion abstraction.

These results demonstrate that the purified ether di-*O*-

methylglycerols are, in particular, composed of a mixture of 1-*O*-hexadec-9-enyl-di-*O*-methylglycerol or [16:1(ω -7)] and of 1-*O*-octadec-9-enyl-di-*O*-methylglycerol or [18:1(ω -9)].

These experiments confirmed results observed by GC/MS/EI of 1-*O*-dimethoxymonoalkyl-di-*O*-methylglycerol derivatives. As is shown on Figure 3, two characteristic fragments of the double-bond position appeared on EI mass spectra of tetramethoxylated 16:0 and 18:0. The ion at m/z 275, namely $CH_3O-CH_2-CHOCH_3-CH_2-O-(CH_2)_8-CH^+-OCH_3$ for both compounds, and ions at m/z 129 or $CH_3O-CH^+-(CH_2)_5-CH_3$ and 157 or $CH_3O-CH^+-(CH_2)_7-CH_3$, respectively, for 16:0 and 18:0, demonstrate that the original double bond was in the Δ 9 position (or ω -7 for 16:1 and ω -9 for 18:1).

Two GC/MS methods were utilized satisfactorily to fix the ethylenic bond position of derivatized 1-*O*-monoalkenylglycerols isolated from the liver oil of the shark *Centrophorus squamosus*: the first procedure consisted of GC/MS/EI of 1-*O*-dimethoxymonoalkyl-di-*O*-methylglycerols derivatives, and the second made use of soft CI with NO on 1-*O*-monoalkenyl-di-*O*-methylglycerols. Both methods have shown that the 16:1 was hexadec-9-enylglycerol and the 18:1 was octadec-9-enylglycerol, namely selachyl alcohol, major components of the unsaturated glycerol ether lipid fraction of deep-sea shark liver oils (19). Considering their natural origin, these glycerol ether lipids should probably be the *Z* iso-

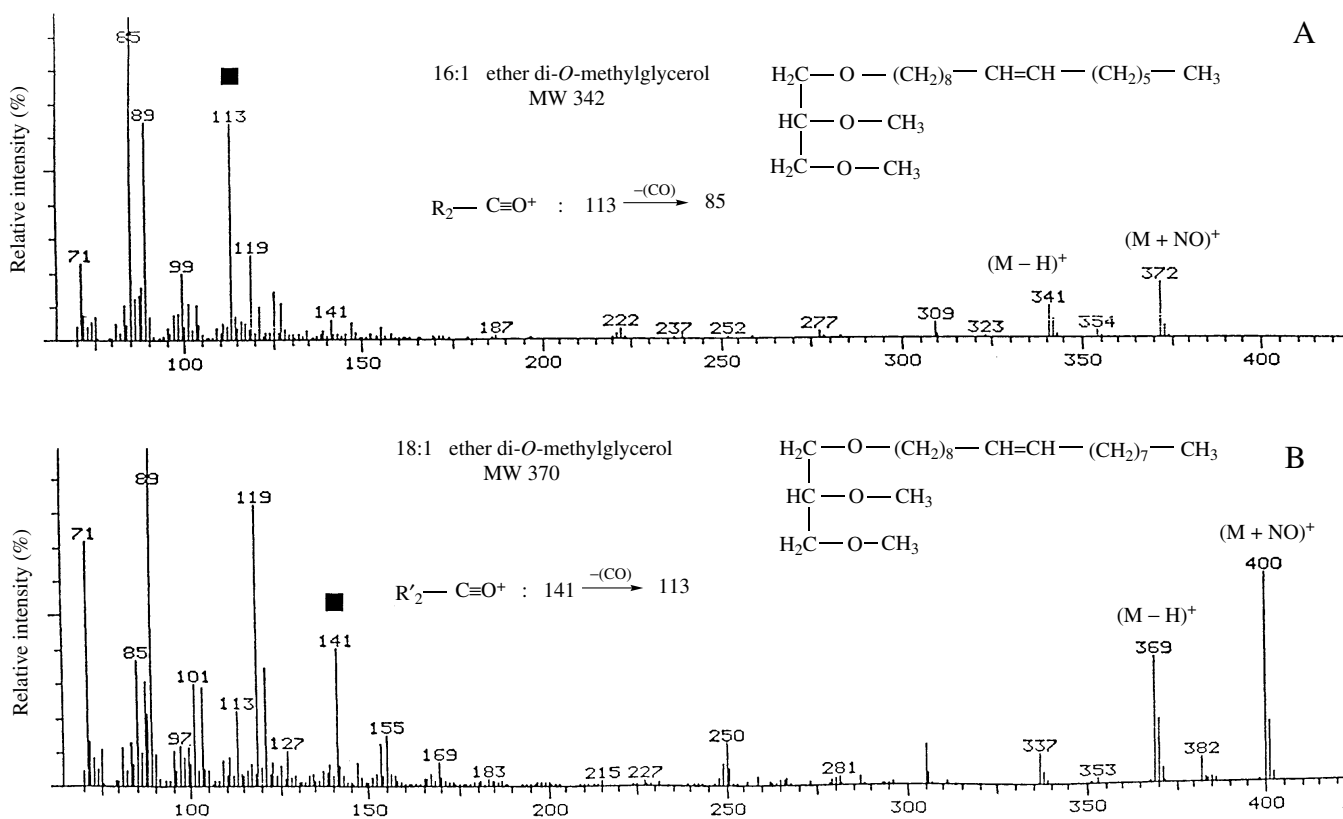


FIG. 2. Nitric oxide chemical ionization mass spectra of 1-*O*-monoalkenyl-di-*O*-methylglycerols: (A) 1-*O*-monohexadec-9-enyl-di-*O*-methylglycerols or 16:1(ω -7); (B) 1-*O*-mono-octadec-9-enyl-di-*O*-methylglycerols or 18:1(ω -9); ■: odd-mass diagnostic ion.

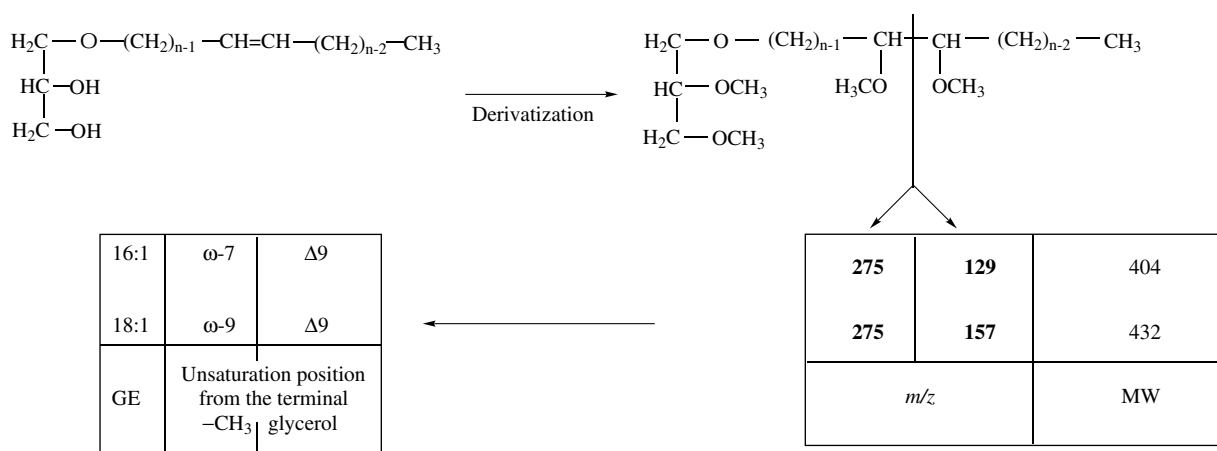


FIG. 3. Position of the double bond of 16:1 and 18:1 from the liver oil of *Centrophorus squamosus* detected by gas chromatography/mass spectrometry/electronic impact after derivatization with OsO₄ and CH₃I; GE, glycerol ether.

mers, but to confirm the double-bond geometry, authentic isomer mass spectra will have to be obtained under exactly the same conditions (4).

This work also demonstrates the suitability of NO⁺ as reactant gas for the determination of double-bond positions of 1-*O*-monoalkenylglycerols.

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