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Mass spectrometric elucidation of chlorine location in dichloro fatty acids following 4,4-dimethyloxazoline derivatization, and its application to chlorinated fatty acids in fish

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Abstract

Fatty acids containing two vicinal chlorine atoms located between C-5 and C-15 were transformed to 4,4-dimethyloxazoline (DMOX) derivatives, which were subjected to electron-impact mass spectrometry (EIMS). The results showed that DMOX derivatization is a useful technique for MS structural analysis of dichloro fatty acids. In contrast to those commonly used derivatives such as alkyl esters which result in mass spectral features indistinguishable among positional isomers, DMOX derivatives of dichloro fatty acids display some distinctive EIMS features which are characteristic of positional isomers. Fragmentation mechanisms responsible for important fragment ions and patterns are inherently related to the position of the vicinal dichloro group on the acyl chain. Among those diagnostic ions and patterns, a homologous series of characteristic chlorodienyl ions, notable by their sizeable intensity and chlorine isotope pattern, are the most valuable structural indicators. The location of the vicinal chlorines in a chlorinated fatty acid can be readily deduced from these ions by equating the mass of the first ion in the series to "144 + 14x", where x represents the location of the first of the vicinal chlorine atoms. In contrast to the "12-mu" rule which, as in the case of monoenoic acids, is only applicable to molecules containing the dichloro group in the central region of the acyl chain, the "144 + 14x" rule is applicable to almost all the positional isomers of vicinal dichloro fatty acids. Using this technique, the 5,6-location of chlorine atoms was confirmed for the previously identified threo-5,6-dichlorotetradecanoic acid in a fish lipid sample.

Keywords: Dichloro fatty acid; Chlorine position; DMOX derivatization; EIMS; Lipid analysis

1. Introduction

Chlorinated fatty acids, typically vicinal dichloro fatty acids, are known to be major components of extractable organochlorine in biota [1–4], probably present in the form of chlorinated acylglycerols. However, the position of chlorine atoms in most of the identified compounds is rather ambiguous, and subject to speculation. Methyl esters are a common type of derivative used in studying chlorinated fatty acids; however, in structural analysis using mass spectrometry, they are vulnerable to chlorine loss with concomitant formation of double bonds. As in the case of unsaturated fatty acids, the location of the chlorine in methyl esters cannot be determined because of double bond ionization and migration.

Pyrrolidides, picolinyl esters, 4,4-dimethyloxazoline (DMOX) derivatives have been the most valuable for locating double bonds in unsaturated fatty acids [5–7]. The principle of using these N-containing derivatives is that nitrogenous functions of the derivatives are highly favourable charge-sites and therefore are subject to preferred ionization, thus minimizing double bond ionization and migration. Picolinyl esters are thought to be better than pyrrolidides on account of the former giving much higher relative abundance of the diagnostic fragment ions [6]. DMOX derivatives give comparable relative abundance of the diagnostic ions and, in addition, have a more desirable GC property: they are usually more volatile than the other two types of derivatives [7]. Another appealing aspect of DMOX derivatives

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is that their preparation is relatively simple and can proceed from various forms of fatty acids as are present in the sample: free fatty acids (using one step of reaction at high temperature [8] or two steps of reactions under much more gentle conditions [8,9]), methyl esters [10] or intact lipids [11].

Åkesson-Nilsson [12] explored the MS structural analysis of chlorinated fatty acids using alkyl esters and pyrrolidides. This was the only reported work directed to examining the feasibility of determining the location of the chlorine in chlorinated fatty acids. Not surprisingly, the alkyl esters yielded no information about the position of chlorine atoms in the molecules. The pyrrolidides gave patterns of fragment ions similar to those obtained from the corresponding monoenoic acid pyrrolidides, but much more complicated. The relative abundance of the diagnostic ions was two-fold lower than that of the corresponding monoenoic acid pyrrolidides. Therefore, it was desirable to find an alternative derivative more suitable for structural elucidation of dichloro fatty acids.

Examining the utility of DMOX derivatives is a logical choice. DMOX, a type of derivative used for protecting the carboxylic acid group in organic synthesis, was first utilized by Huang and co-workers in late 1980s for locating double bonds [13–15], triple bonds [16], methyl branching [8], cyclopropane rings [17], cyclopentene rings [18] and oxygenated groups [19,20] in fatty acids. Later this technique was adopted by other researchers and extended to additional types of structural elements in fatty acids, for instance, the cyclopropene ring [21]; cyclopetane, cyclohexane, cyclohexene and diunsaturated six-membered rings [22]; and the oxirane ring [23]. In this study, utilizing DMOX derivatives for MS structural analysis of dichloro fatty acids was evaluated. Possible fragmentation pathways for important ions were proposed and a "144 + 14*x*" rule was identified: the first in a series of chloro ions has a *m/z* equal to "144 + 14*x*", where *x* is a number corresponding to the location of the first of the vicinal chlorine atoms on the acyl chain. As demonstrated in the paper, this rule can be used for quickly locating the chlorine in vicinal dichloro fatty acids, and the confirmation can then be made by other characteristic fragment ions and patterns known for that dichloro location. Application of this technique was illustrated by the confirmation of *threo*-5,6-dichlorotetradecanoic acid previously identified in a fish lipid sample [24].

2. Experimental

2.1. Synthesis of chlorinated fatty acids

A series of chlorinated fatty acids, *threo*-5,6-, *threo*-9,10- and *erythro*-9,10-dichlorotetradecanoic acids, *threo*-9,10- and *erythro*-9,10-dichlorohexadecanoic acids, and *threo*-6,7-, *threo*-9,10-, *erythro*-9,10-, *threo*-11,12-, *threo*-12,13-, *threo*-13,14- and *threo*-15,16-dichlorooctadecanoic acids, were prepared by chlorination of the corresponding *cis*- and *trans*-monoenoic acids. All the monoenoic acids except *cis*-5-tetradecenoic acid were purchased from Aldrich. The latter was synthesized using a procedure based on Jin and Tserng [25]: condensation of 5-chloro-1-pentyne with 1-iodooctane, condensation of the resulting product with potassium cyanide, hydrolysis, and hydrogenation. Details of these preparations were given previously [24,26].

2.2. Fish sample preparation

Ground filets of white sucker, sampled downstream from a bleached kraft mill, were extracted with hexane:acetone (3:1) consecutively at 55 and 100 °C. The extract was washed with nanopure water and separated into two fractions by gel permeation chromatography. The higher molecular weight fraction (approx. >350 Da), presumably composed mainly of lipids, were transformed to fatty acid methyl esters (FAMEs) by acidic methanolysis. The chlorinated FAMEs were enriched by HPLC fractionation. The experimental details were reported previously [26,27].

2.3. 4,4-Dimethyloxazoline derivatization

Free fatty acid (FA) or FAME (2–4 mg) was dissolved in 2-amino-2-methyl-1-propanol (100–200 mg) in a capped vial flushed with nitrogen. The mixture was heated (the vial was re-flushed when it was heated to ∼100 °C) and kept at about 170–190 °C for 2 h for free fatty acid [8] and 18 h for FAME [28]. Upon cooling, diethyl ether:cyclohexane (1:1) and nanopure water (3 ml each) were added, and the vial was shaken. The organic phase was further washed with 2 × 3 ml of nanopure water, dried over anhydrous sodium sulphate and passed through fresh anhydrous sodium sulphate packed in a Pasteur pipette. The filtrate was evaporated to dryness in a stream of nitrogen.

2.4. Gas chromatography/mass spectrometry

The GC/MS used was an HP 5890 II GC coupled to a VG Trio-1000 MS (using a quadrupole as the mass analyzer). The sample, dissolved in cyclohexane, was injected in the splitless mode (injector temperature 250 °C) and eluted through a fused silica capillary column (DB-1, J&W Scientific), 30 m × 0.25 mm × 0.25 μm, using H_2 (1.8 ml/min) as the carrier gas. For elution of standards, the oven temperature was initially kept at 100 °C for 1 min, then increased to 280 °C at a rate of 25 °C/min, where it was held for 5 min. The retention times of the standards under these GC conditions are listed in Table 1. For the fish sample preparation, the oven temperature was initially kept at 100 °C for 1 min, raised to 200 °C at 25 °C/min, then to 240 °C at 4 °C/min and finally to 300 °C at 25 °C/min, where it was held for 2 min. Electron-impact ionization with an electron energy of 70 eV and a source temperature of 200 °C was used. The solvent delay was 3 min.

Table 1
Retention time (RT) of synthesized standards under chromatographic conditions used in this study

DMOX derivative of	RT (min)
threo-5,6-Dichlorotetradecanoic acid	7.56
threo-9,10-Dichlorotetradecanoic acid	7.71[a]
threo-9,10-Dichlorohexadecanoic acid	8.44
threo-6,7-Dichlorooctadecanoic acid	9.10
threo-9,10-Dichlorooctadecanoic acid	9.13
threo-11,12-Dichlorooctadecanoic acid	9.15
threo-12,13-Dichlorooctadecanoic acid	9.20
threo-13,14-Dichlorooctadecanoic acid	9.22
threo-15,16-Dichlorooctadecanoic acid	9.26
erythro-9,10-Dichlorotetradecanoic acid	7.66
erythro-9,10-Dichlorohexadecanoic acid	8.41[a]
erythro-9,10-Dichlorooctadecanoic acid	9.09
cis-5-Tetradecenoic acid	6.85
cis-9-Tetradecenoic acid	6.93
cis-9-Hexadecenoic acid	7.74[a]
cis-6-Octadecenoic acid	8.47
cis-9-Octadecenoic acid	8.50
trans-9-Tetradecenoic acid	6.89
trans-9-Hexadecenoic acid	7.71
trans-9-Octadecenoic acid	9.76[a]

[a] GC-MS was done on a different day, on which the carrier flow could be slightly different.

3. Results and discussion

3.1. General features of mass spectra of DMOX derivatives of dichloro fatty acids

Mass spectra of the DMOX derivatives of dichloro fatty acids are very similar to those of corresponding monoenoic acids from which dichloro fatty acids can be made by chlorination. Fig. 1 shows the spectra of DMOX derivatives of *cis*-5-tetradecenoic, *threo*-5,6-dichlorotetradecanoic, *cis*-6-octadecenoic, *threo*-6,7-dichlorooctadecanoic, *cis*-9-octadecenoic and *threo*-9,10-dichlorooctadecanoic acids, and the 11,12-, 12,13-, 13,14- and 15,16-dichloro positional isomers of the DMOX derivative of *threo*-dichlorooctadecanoic acid (the spectra of all positional isomers of the DMOX derivative of octadecenoic acid can be found in the literature [13,29] and are readily available in a website hosted by Christie [30]). As it can be seen from these mass spectra and those shown in [30], there are many similarities between DMOX derivatives of dichloro fatty acids and their monoenoic analogues. It is an advantage to show the spectra of the DMOX derivatives of dichloro fatty acids along with their monoenoic analogues. Before we present characteristic features of the positional isomers of the dichloro fatty DMOX derivatives we have studied, it is desirable to summarize their general spectral features, which appear to be similar even to those of DMOX derivatives of fatty acids of other kinds.

First of all, fragment ions in the mass range below *m/z* 113 are neither abundant nor informative and thus are excluded for consideration in structural analysis. An ion either at *m/z* 113 or at *m/z* 126 is usually the base peak (exceptions are those that have a double bond at C-4 or closer to the heterocyclic end [30]). It is well accepted that the *m/z* 113 ion is formed by the McLafferty rearrangement. The *m/z* 126 ion was previously thought as resulting from the cyclization-displacement reaction [7,13]: a homolytic cleavage of a σ bond between C-3 and C-4 is induced by the charge site at N with a concomitant closure between C-3 and N to form a four-member ring, which does not seem to be energetically favoured. Indeed, in a recent study of the DMOX preparation of [$^2H_{35}$] octadecanoic acid, Hamilton and Christie [31] disproved the cyclization-displacement and suggested that the *m/z* 126 ion is actually an ion with conjugated double bonds, derived from a more complex but energetically more favoured process involving distonic ions as intermediates.

Though the relative intensity of the *m/z* 113 and 126 ions can be an important feature for distinguishing some positional isomers, much of the structural information is obtained from less abundant fragment ions in the mass range above *m/z* 126. As in the case of other N-containing derivatives, the formation of these ions is generally attributed to simple radical-induced cleavage [7]: a hydrogen atom from every position of the chain tends to migrate to the charge centre; the resulting radical site causes fission of a carbon-carbon bond with concomitant formation of a π bond. This mechanism results in a homologous series of ions with an increment of normally 14 mu. Recently, Christie and co-workers [29,31] showed that the elimination of a methyl radical, which gives the last member in the series ($[M-15]^+$), occurs almost exclusively on the DMOX ring rather than on the carbon chain. The series would be interrupted at a point where a functional group is present on the carbon chain. Besides, any large variation in abundance between these homologous ions is also an indication of the presence of a functional group. Another fragmentation mechanism that is common to DMOX derivatives may involve expulsion of a segment between C-2 and C-3 or C-4 as an ethyl or propyl radical [31], giving rise to ions of $[M-29]^+$ and $[M-43]^+$, which would not fit into the fore-mentioned homologous series if a functional group were located between C-2 and C-4.

3.2. Dichloro atoms at C-5 and C-6 in comparison of its monoenoic analogue

As can be seen in Fig. 1a, the most striking fragment pattern of the DMOX derivative of *cis*-5-tetradecenoic acid is two intense peaks at *m/z* 180 and 153, respectively, and a small intensity ratio of the *m/z* 126 peak to the *m/z* 113 peak. The ion at *m/z* 180 is apparently derived from an energetically favoured fragmentation, an allylic cleavage between C-7 and C-8 induced by abstraction of an allylic hydrogen atom at C-4 to the ionized nitrogen atom, and thus has a greater abundance than other members in the homologous series. The formation of the odd-numbered ion at *m/z* 153 probably involves a mechanism (Fig. 2) similar to the one proposed by Fay and Richli [10] for an ion at *m/z* 139 in the mass spectrum of the DMOX derivative

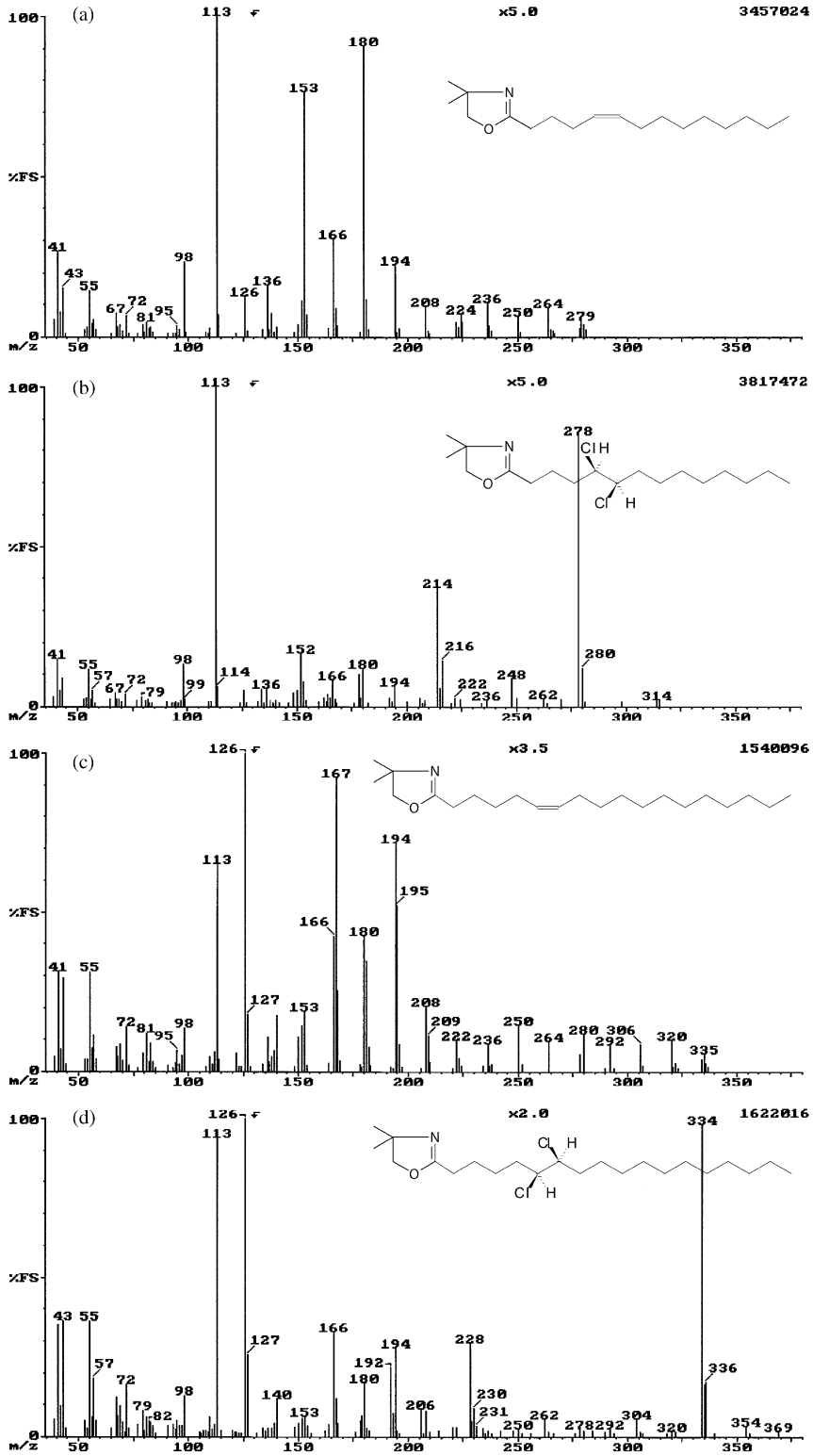

Fig. 1. EI mass spectra of 4,4-dimethyloxazoline derivatives of some fatty acids. (a) *cis*-5-Tetradecenoate, (b) *threo*-5,6-dichlorotetradecanoate, (c) *cis*-6-octadecenoate, (d) *threo*-6,7-dichlorooctadecanoate, (e) *cis*-9-octadecenoate, (f) *threo*-9,10-dichlorooctadecanoate, (g) *threo*-11,12-dichloro-octadecanoate, (h) *threo*-12,13-dichlorooctadecanoate, (i) *threo*-13,14-dichlorooctadecanoate, and (j) *threo*-15,16-dichlorooctadecanoate. The number of counts for the base peak is given in the upper right concern of each spectrum. In (e)–(j), a diagnostic 12-mu difference between two neighbouring ions is indicated by a bracket with the number 12.

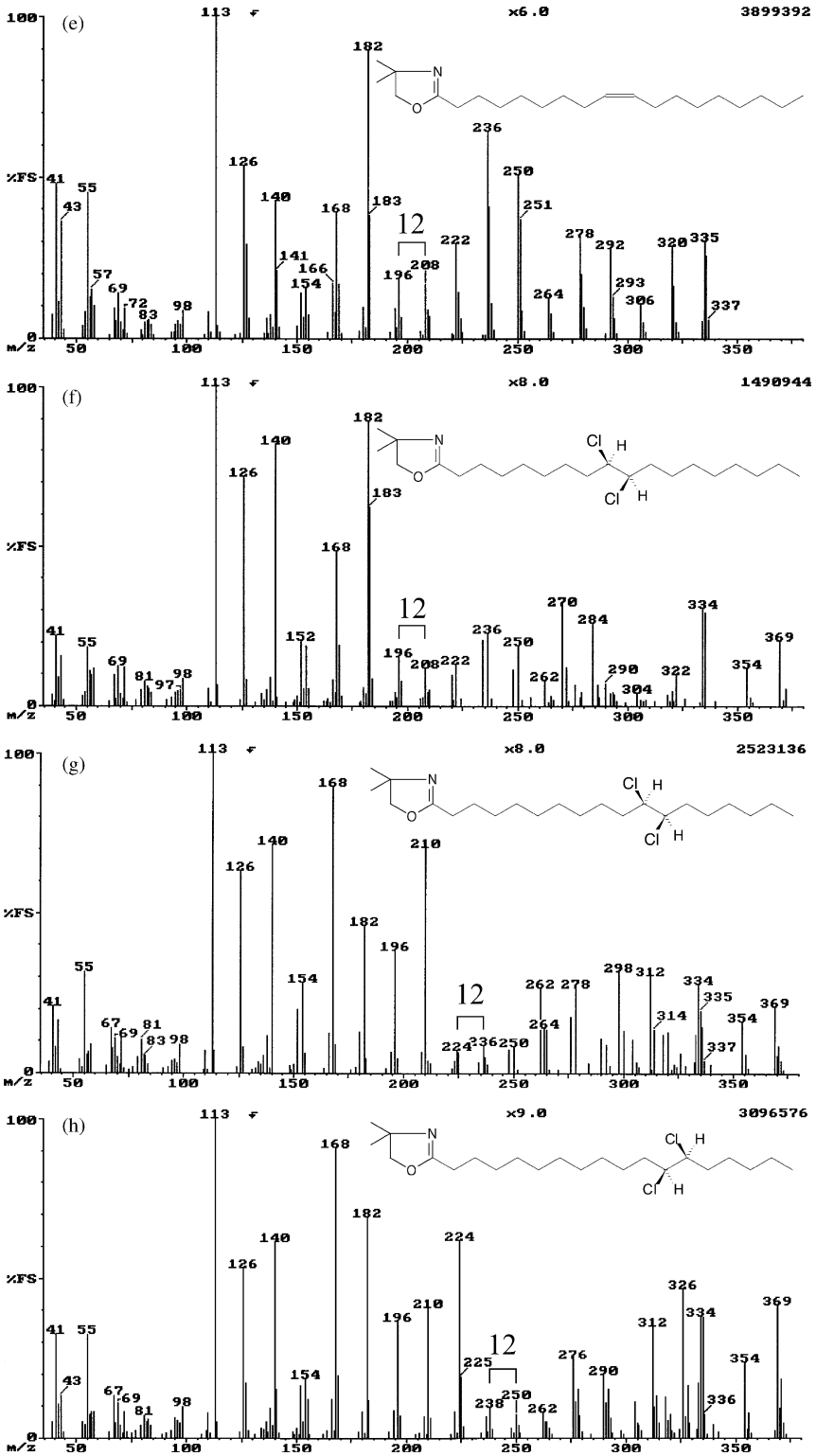

Fig. 1. (*Continued*)

of the 22:6(4,7,10,13,16,19) fatty acid. As we can see in Fig. 2, the pathway (B) plays an important role in minimizing the migration of the occasionally ionized double bond. Compared to other positional isomers, the DMOX derivative of 5-monoenoic acid displays a particularly high intensity at *m/z* 113 (base peak) relative to other peaks. This is expected from the thermodynamic perspective: the McLafferty rearrangement of this isomer results in not only a stable McLafferty rearrangement ion but also a stable leaving group (a conjugated diene).

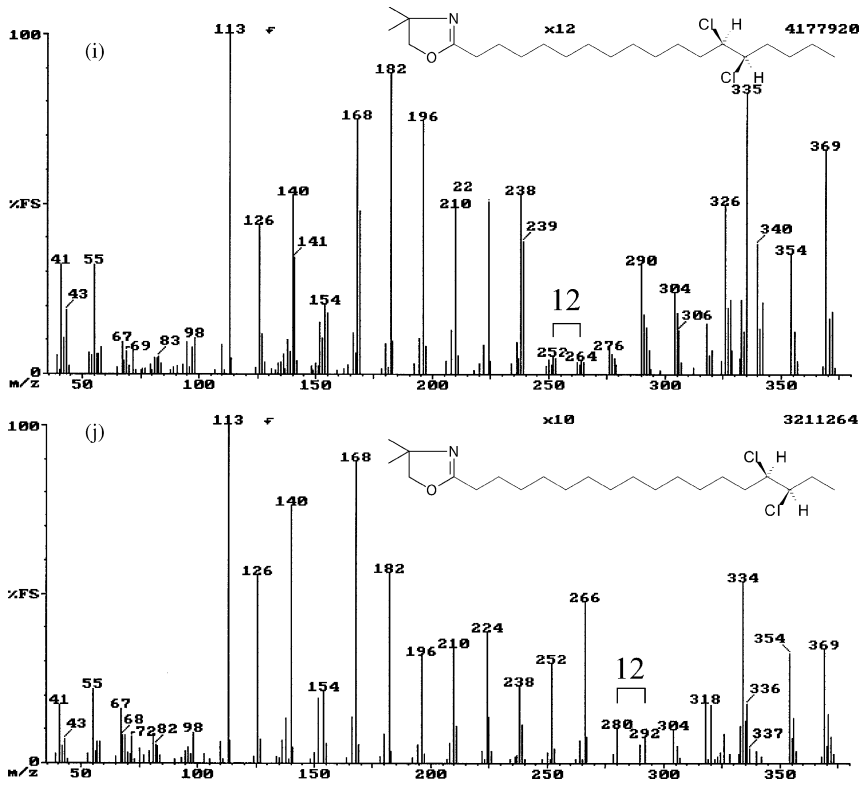

Fig. 1. (*Continued*).

Part of the mass spectrum of the DMOX derivative of *threo*-5,6-dichlorotetradecanoate (Fig. 1b) is quite similar to that of the DMOX derivative of 5-tetradecenoic acid, showing corresponding fragment ions and an exceptionally small intensity ratio of the *m/z* 126 peak to the *m/z* 113 peak. But the odd-numbered ion at *m/z* 153 is not as abundant as the even-numbered ion at *m/z* 152. The relative increase in the abundance of the *m/z* 152 ion may be attributed to a loss of HCl with concomitant formation of a double bond between C-4 and C-5, which then undergo a cleavage in a way as depicted by Christie et al. [29] for the same ion in the spectrum of the DMOX derivative of 4-octadecenoic acid. Nevertheless, the presence of important diagnostic ions resembling those from the monoenoic analogue suggests that a major pathway of losing chlorine from DMOX derivatives of fatty acids containing two vicinally positioned chlorine atoms may be a removal of Cl_2 with concomitant formation of a double bond between the carbon atoms originally bonded with the chlorine atoms.

Fig. 1b contains additional ions which are not present in the spectrum of the monoenoic analogue. The ions at *m/z* 214 and 216 are apparently isotopes containing one Cl atom. This indicates the presence of a chlorodienyl structure, resulting from a loss of HCl which gives rise to a chloroethenylene structure (–CH=CCl–) in the carbon chain and a subsequent allylic cleavage which leads to a conjugated chlorobutadienyl structure (–CH=CH–CCl=CH_2 or –CH=CCl–CH=CH_2). The proposed mechanism is shown in Fig. 3. As we will see in the spectra of DMOX derivatives of other dichoro fatty acids, this kind of conjugated chlorobutadienyl ions is specific to the vicinal dichloro position since its *m/z* is a function of *x*, i.e. $144 + 14x$, where *x* represents the location of the first of the two vicinally positioned chlorine atoms on the carbon chain. For example, *x* is 5 for 5,6-dichloro fatty acids. The proposed mechanism predicts that this $144+14x$ formula should hold for $2 < x < n-2$, where *n* is the length of the acyl chain. There is a very weak peak at *m/z* 298, which is probably derived from a loss

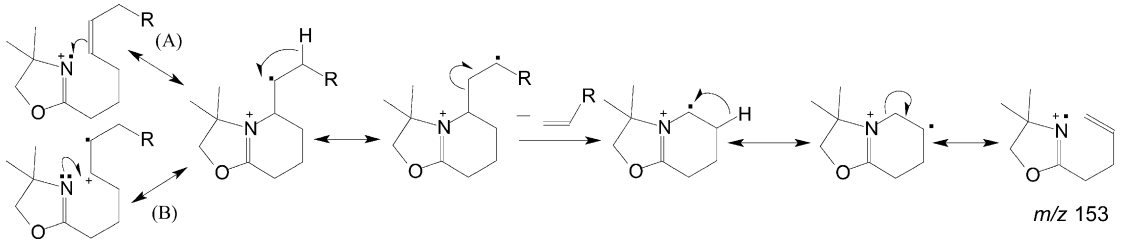

Fig. 2. Possible fragmentation mechanism for the formation of the ion at *m/z* 153 in EIMS of the DMOX derivative of 5-monoenoic fatty acid.

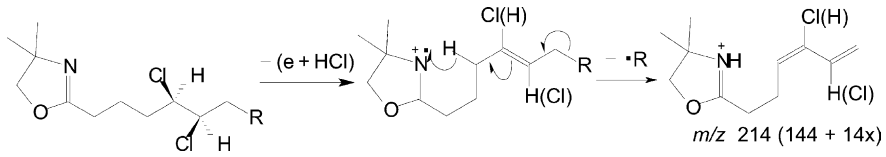

Fig. 3. Proposed fragmentation mechanism for the formation of conjugated chlorobutadienyl ions ($144 + 14x$) in EIMS of the DMOX derivative of fatty acid containing two chlorine atoms at C-5 and C-6.

of HCl and methyl radical. The mechanism of the methyl loss may be the same as in the case of monoenoic analogue where the loss of a methyl radical from the molecular ion of the DMOX derivative occurs on the heterocyclic structure [30,31] rather than at the end of the carbon chain. It follows that, instead of having a 4,4-dimethyloxazoline ring plus an chloroethenylene structure with an unconjugated ethenyl group ending the chain, the *m/z* 298 ion probably contains a 4-methyloxazole ring and a chloroethenylene element embodied in the carbon chain. Its expected isotopic ion at *m/z* 300 is not discernible because of low intensity. A relatively intense peak at *m/z* 278 is a useful diagnostic indicator. This ion is likely $[M - HCl - Cl]^+$, which may be a result from energetically (maybe kinetically as well) favoured dechlorinations (Fig. 4). Note that in pathway (A) an allylic hydrogen atom is readily positioned to favour its abstraction. As will be found in other positional isomers later, $[M - HCl - Cl]^+$ is no longer prominent when vicinal chlorine atoms are positioned farther away from the heterocyclic end.

There is a series of weak ions (from *m/z* 178 to 262 with an increment of 14 mu) which are 2 mu lighter than those normal diagnostic ions (of monoenoic origin). The presence of these ions indicates that dechlorination involving a loss of two HCl molecules occurs with concomitant formation of a conjugated dienyl structure where one double bond is present between C_{x-1} and C_x and the other between C_{x+1} and C_{x+2}. It is conceivable that any cleavage that retains the butadienylene structure in the charged fragment will lead to a "2 mu lighter" ion (trienyl). The relatively intense ion at *m/z* 178 may be explained by formation of a cyclic conjugated structure resulting from a mechanism illustrated in Fig. 5, part of which is analogous to that proposed in [29] for the *m/z* 152 ion from the DMOX derivative of 4-octadecenoic acid. In the heavier portion of this series, the abundance of these "2 mu lighter" ions (*m/z* 248 and 262) exceeds that of "normal" ions in the spectrum. The *m/z* 262 ion probably contains a 4-methyloxazole ring and a conjugated dienyl chain. The formation of the *m/z* 248 ion may be caused either by hydrogen abstraction at C-12 which may have been oriented to a favourable position or by a cyclization followed by expulsion of an ethyl radical as depicted in Fig. 6.

It was noted that there was an accompanying compound in the DMOX preparation of *threo*-5,6-dichlorotetradecanoic acid. In the GC/EIMS, it eluted earlier than the DMOX derivative of *threo*-5,6-dichlorotetradecanoic acid, and its EI mass spectrum had the base peak at *m/z* 113 and a strong

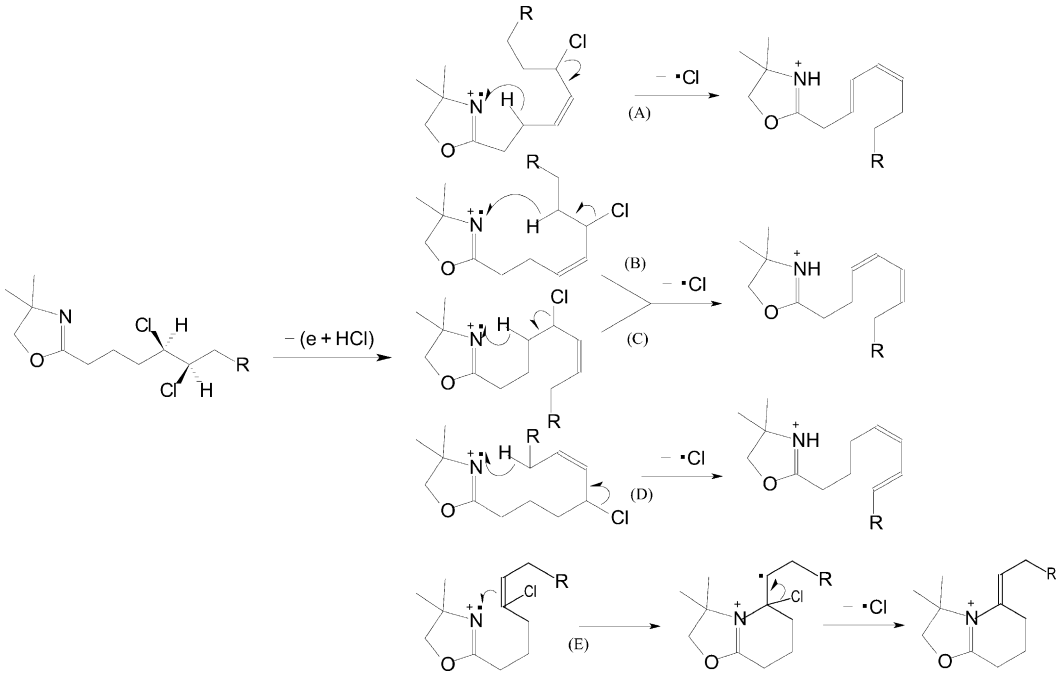

Fig. 4. Possible fragmentation pathways leading to the formation of ions containing conjugated butadienylene or cyclic structure (*m/z* 278) in EIMS of the DMOX derivative of 5,6-dichlorotetradecanoic acid ($R = C_7H_{15}$). Note that the intermediates in pathways (A) and (B) are identical, and the same is true for pathways (C) and (D).

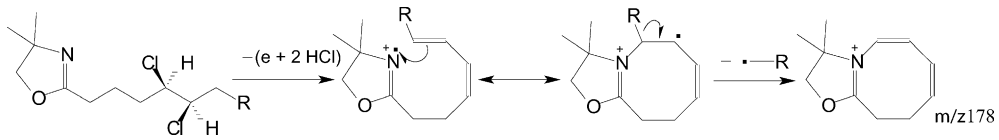

Fig. 5. Possible fragmentation mechanism for the formation of the *m/z* 178 ion in EIMS of the DMOX derivative of fatty acid containing two chlorine atoms at C-5 and C-6.

peak at *m/z* 278 but very weak peaks at *m/z* 152 and 214. The origin of this compound is not clear.

3.3. Dichloro atoms at C-6 and C-7 in comparison of its monoenoic analogue

A mass spectrum of the DMOX derivative of *cis*-6-octadecanoic acid was published previously by Zhang et al. [13]. A similar spectrum obtained in this study is shown here (Fig. 1c) to facilitate comparison between 6-monoenoic and 6,7-dichloro analogues. As we see, the distinctive features for the double bond at C-6 include the base peak at *m/z* 126 instead of 113, a strong peak at *m/z* 167 and abundant allylic cleavage ions at *m/z* 194. The fragmentation mechanism for the formation of the odd-numbered ion at *m/z* 167 is analogous to the one shown in Fig. 2. The formation of the *m/z* 194 ion involves the same mechanism as for the *m/z* 180 ion in the spectrum of its 5-enoic isomer.

The mass spectrum of the DMOX derivative of *threo*-6,7-dichlorooctadecanoic acid in Fig. 1d has a very similar fragmentation pattern in the range below *m/z* 335. Some major differences include even-numbered ions at *m/z* 166 which are more abundant than the odd-numbered ions at *m/z* 167 (the reason for this is the same as for the DMOX derivative of 5,6-dichlorotetradecanoic acid described previously), distinctive conjugated chlorobutadienyl ions at *m/z* 228 ($144 + 14x$), chloroethenylene ions of 2-methyloxazole type at *m/z* 354, a series of "2 mu lighter" ion peaks differing by 14 mu from *m/z* 178 to 318 (with peaks at *m/z* 192, 206, 262 and 304 showing more appreciable intensity), and an intense $[M - HCl - Cl]^+$ peak (*m/z* 334).

3.4. Dichloro atoms in the middle of the acyl chain in comparison of the corresponding monoenoic analogues

It has been well established that in the EIMS of the DMOX derivative of an unsaturated fatty acid with the double bond in the middle of the carbon chain, say, between C_x and C_{x+1}, a 12-mu interval instead of 14-mu occurs between ions derived from cleavages of C_{x-1}–C_x and C_x–C_{x+1}. Indeed, this "12-mu" rule applies perfectly to the double bond located anywhere between C-8 and C-15 in the DMOX derivatives of the 18:1 fatty acids [30]. Apparently, a series of ions with an increment of 14 mu present before the 12-mu interval are of monoenyl or cyclic carbon chain, and after the interval they are of dienyl type (including the cyclic monoenyl carbon chain and the monoenyl carbon chain with a methyloxazole ring). The C_x–C_{x+1} cleavage, resulting in an ion of dienyl or cyclic monoenyl type, may have occurred as a result of the double bond ionization and subsequent migration in the heterocyclic direction. The intensity of this peak is low, reflecting the fact that the degree of double bond migration is very low in DOMX derivatives.

In many cases, there are additional clues for structural elucidation: more intense peaks arising from allylic cleavages (between C_{x-2} and C_{x-1} and between C_{x+2} and C_{x+3}) and sometimes a cleavage between C_{x+3} and C_{x+4} induced by the abstraction of an allylic H atom on the chain terminus side. Thus, a 12-mu interval is easily identified or confirmed as a local valley is present in the spectrum with three low abundant ions derived from cleavages of C_{x-1}–C_x, C_x–C_{x+1} and C_{x+1}–C_{x+2} being flanked with three more intense peaks arising from energetically favoured allylic cleavages and C_{x+3}–C_{x+4} cleavage.

In the mass spectrum of the DMOX derivative of *cis*-9-octadecenoic acid shown in Fig. 1e, which is similar to those published in [13,30], a 12-mu interval is readily seen between peaks at *m/z* 196 and 208 as they are flanked with more intensive peaks at *m/z* 182, 236 and 250. The mass spectrum of the DMOX derivative of *threo*-9,10-dichlorooctadecanoic acid in Fig. 1f has all fragment ions appearing in its monoenoic analogue, differing only in relative abundance. The most remarkable similarity is that there is a 12-mu interval present in the same location. The left-side flanking peak (*m/z*

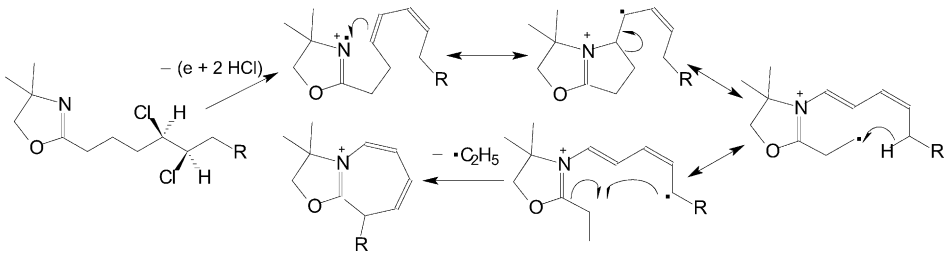

Fig. 6. Possible fragmentation mechanism for the formation of the *m/z* 248 ion in EIMS of the DMOX derivative of 5,6-dichlorotetradecanoic acid ($R = C_7H_{15}$).

182) is very strong; the two right-side flanking peaks (*m/z* 236 and 250), though not so prominent, are still more intense than the 12-mu interval peaks (*m/z* 196 and 208). The ions characteristic of the 9,10-dichloro location include a series of 14-mu interval peaks of chlorodienyl ions (resulting from a loss of HCl and subsequent fragmentation) starting from *m/z* 270 and ending at *m/z* 354, a series of "2 mu lighter" peaks of trienyl ions starting from *m/z* 220 with a 14-mu increment, and some moderate peaks at *m/z* 334 ($[M - HCl - Cl]^+$), 335 ($[M - 2Cl]^{\bullet+}$) and 369 ($[M - HCl]^{\bullet+}$). Interestingly, the latter two are not seen in Fig. 1b of the 5,6-dichloro isomer, very week in Fig. 1d of the 6,7-dichloro isomer, and strong in Fig. 1f–j of positional isomers having vicinal chlorine atoms at C_9 and C_{10} or farther from the heterocyclic end. The chlorine isotope pattern is clearly observed for $[M - HCl]^{\bullet+}$ (peaks at *m/z* 369 and 371). The radical ions $[M - HCl]^{\bullet+}$ (either chloroethenylene or chloropropenylene (–CH=CH–CHCl–) structure) and $[M - 2Cl]^{\bullet+}$ probably result from a loss of HCl and Cl_2, respectively, from the molecular ion without further fragmentation. The *m/z* 270 and 284 ions with comparable intensities are chlorodienyl ions in which two double bonds are conjugated, and are thus more abundant than unconjugated members in the series. Apparently, these two ions are derived from two energetically favoured fragmentations of a chloroethenylene ion that has resulted from a loss of HCl (Fig. 7). The *m/z* 270 ion arises from an allylic cleavage as previously discussed ($144+14x$), while the *m/z* 284 ion arises from a cleavage induced by an allylic hydrogen abstraction (at C_{x+2}). Interestingly, the latter type of cleavage does not happen in the fragmentation of the DMOX derivative of 5,6-dichlorotetradecanoic acid since the *m/z* 228 ion is not present in its mass spectra (Fig. 1b). The presence of only a trace peak at *m/z* 242 in Fig. 1d indicates that this cleavage barely occurs in the fragmentation of the DMOX derivative of 6,7-dichlorooctodecanoic acid. Thus, it appears that the abstraction of the allylic hydrogen at C_{x+2} is sterically hindered by the neighbouring bulky chlorine atom, which seemingly becomes more influential when the rigid chloroethenylene group moves closer to the heterocyclic group. An additional reason could be that this abstraction is competed by the abstraction of the allylic hydrogen at C_{x-1} which is sterically far more favoured when C_{x-1} is located closer to the charge centre.

The mass spectra of the DMOX derivatives of additional monoenoic acids with double bond at C_9 and 9,10-dichloro fatty acids were studied to examine the spectral differences between *cis*- and *trans*-isomers of monounsaturated fatty acids, between *threo*- and *erythro*-isomers of dichloro fatty acids, and between different lengths of the acyl chain. Comparison of these derivatives shows that there are no distinct fragment ions or patterns that can be used for distinguishing between *cis*- and *trans*-isomers or between *threo*- and *erythro*-isomers. Different chain lengths do not alter the features of diagnostic ions that are characteristic of that double bond or vicinal chlorine location.

Shown in Fig. 1g–j are the mass spectra of DMOX derivatives of 11,12-, 12,13-, 13,14- and 15,16-dichloro fatty acids. These spectra contain fragment ions and characteristic pattern that are present in the mass spectra of their monoenoic analogues as illustrated in [30]. The DMOX derivatives of octadecenoates with the double bond at C-11, C-12 and C-13 have the same spectral features as for the DMOX derivative of 9-octadecenoic acid, typified by two ions separated by a 12-mu interval and flanked with three more intense peaks: one on the left-hand side (allylic cleavage) and the other two on the right-hand side (allylic and C_{x+3}–C_{x+4} cleavages). The mass spectrum of the DMOX derivative of 15-octadecenoic acid also has the same features except that there is no C_{x+3}–C_{x+4} cleavage ion since C_{x+4} does not exist in the molecule. In the spectra of the DMOX derivatives of the dichloro fatty acids, in addition to these monoenoic-origin ions, other important ions include a series of 14-mu incremental chlorodienyl ions starting from *m/z* ($144 + 14x$) with the first two peaks being usually the strongest. Ions of $[M - HCl - Cl]^+$ and $[M - HCl]^{\bullet+}$ are moderately intense in all of these spectra. The presence of a "trienyl" type of ions forming a "2 mu lighter" series is also typical of these spectra.

3.5. Summary of characteristic MS features of DMOX derivatives of vicinal dichloro fatty acids

It is evident from a comparison of the foregoing mass spectra that most diagnostic ions in the spectra of DMOX derivatives of dichloro fatty acids are generally slightly lower in the relative (to the base peak) abundance than those

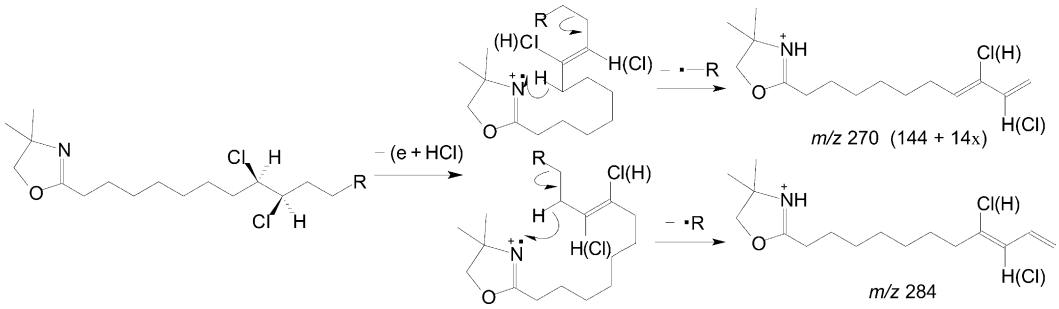

Fig. 7. Proposed fragmentation pathways leading to the formation of conjugated chlorobutadienyl ions in EIMS of the DMOX derivative of fatty acid containing two chlorine atoms at C-9 and C-10.

in the spectra of their monoenoic analogues. Nevertheless, chlorodienyl ions are prominent. These characteristic ions are readily identified not only by their intensity but also by their chlorine isotope pattern. The "144 + 14*x*" formula can be applied to the first ion in the chlorodienyl series for a facile determination of the dichloro location ("144 + 14*x*" rule). For vicinal chlorine atoms residing in the middle of the chain, $[M - HCl]^{\bullet+}$ is also notably abundant. An additional important diagnostic pattern is a local valley characterized by a 12-mu interval and intense flanking ions ("12-mu" rule). For dichloro locations close to the DMOX ring such as 5,6- and 6,7-dichloro locations, the diagnostic ion $[M-HCl-Cl]^+$ displays an exceptionally high intensity, second only to the base peak. Information about the abundance ratio of the *m/z* 113 versus 126 ions and "2 mu lighter" ions seen in the higher mass range also helps in structural elucidation.

In terms of fragmentation mechanism, prominent fragment ions and patterns are inherently related to the position of the vicinal dichloro group on the acyl chain. They are attributable to: (1) a loss of Cl_2 that results in a double bond, which undergoes subsequent fragmentations typical of the DMOX derivatives of monoenoic acids; (2) a loss of HCl that results in chloroethenylene ions, which undergo further fragmentation yielding characteristic chlorodienyl ions the lightest of which is the "144 + 14*x*" ion; (3) a loss of HCl that results in an ion containing a chloropropenylene or cyclic structure, which subsequently loses a chlorine radical to form a conjugated dienyl structure or a cyclic conjugated structure ($[M - HCl - Cl]^+$); and (4) a loss of two HCl and subsequent fragmentations typical of the DMOX derivatives of conjugated dienoic acids that gives rise to a series of "2 mu lighter" fragment ions.

3.6. Application in analysis of a fish sample

An HPLC fraction of the transesterified fish extract was subjected to DMOX derivatization. The GC/EIMS of the resultant DMOX derivatives is presented in Fig. 8. Shown in Fig. 8a is the total ion chromatogram (TIC), where the peak of interest is indicated by an arrow in the inset, which is an enlargement of the framed part in the chromatogram (note that the horizontal dimension has been enlarged more than the vertical dimension). The mass spectrum of this peak is shown in Fig. 8b. The concentration of the analyte was very low in the sample, as can be appreciated by comparison of

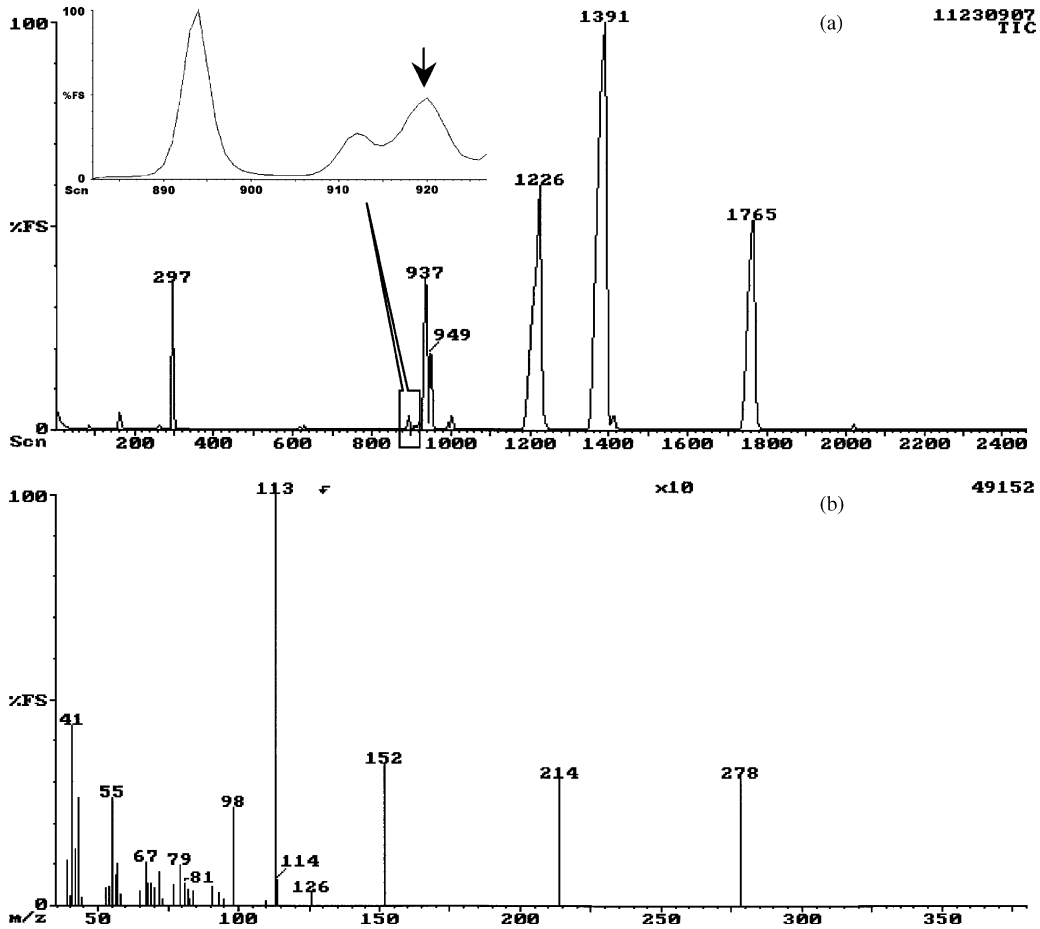

Fig. 8. GC/EIMS of the DMOX derivative of an HPLC fraction of transesterified fish extract. (a) Total ion chromatogram; (b) EI mass spectrum obtained by scanning over the central portion of the peak indicated by an arrow in (a), confirming 5,6-dichlorotetradecanoic acid previously identified in the sample.

the number shown in the upper right concern of this spectrum with the number in the spectrum of the corresponding standard in Fig. 1b (these numbers represent the ion counts for the respective base peaks). It is not surprising that low abundant fragment ions were not seen in the mass spectrum shown in Fig. 8b. Nevertheless, the characteristic peaks in the spectrum resembles those of the standard shown in Fig. 1b, thus providing a direct evidence for the 5,6-location of chlorine atoms in the dichlorotetradecanoic acid present in the fish extract, previously identified by virtue of retention-time match between the analyte and an authentic synthesized compound [24].

4. Conclusions

The 4,4-dimethyloxazoline derivatives can be used with GC/EIMS for locating the chlorine in fatty acids containing vicinal dichloro atoms. Almost all important peaks in the mass spectra are explainable mechanistically as they are inherently related to molecular structure. Abundant ions are associated with fragmentation processes that are energetically favoured and in which the carbon chain is readily oriented to a conformation in favour of interaction between reaction sites. The important diagnostic indicators are a large portion of ions that resembles its monoenoic analogue due to facile loss of Cl_2, accompanied by a portion of "2 mu" lighter ions in the heavier mass range as a result of loss of two HCl; a $[M - HCl - Cl]^+$; and above all, a "$144 + 14x$" ion which is the lightest of those ions displaying a chlorine isotope pattern and can be readily used for deducing dichloro location.

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