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Review

Investigation of aldehydic lipid peroxidation products by gas chromatography–mass spectrometry

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Abstract

Lipid peroxidation (LPO) of polyunsaturated fatty acids is induced in injured or dying cells. The generated lipid hydroperoxides readily decompose to a great variety of aldehydic compounds, which are consequently useful markers for LPO processes in biological materials. Since aldehydes are produced in tiny amounts only, their detection requires efficient separation methods combined with unambigious and specific identification techniques – e.g. separation by gas chromatography followed by identification with electron impact mass spectrometry. A great number of LPO aldehydes contains polar groups, especially carboxylic or hydroxy functions. These groups may cause decomposition when a sample is heated up in the hot injector of a gas chromatograph. Therefore such aldehydes must be protected before analysis. Derivatization enhances also volatility. Advantages and disadvantages of different methods to prepare suitable derivatives of LPO aldehydes for GC separation are discussed. The mass spectra of different derivatives are compared in order to demonstrate which ones are most useful to gain maximum information on the structure of the original aldehydes. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Gas chromatography–mass spectrometry; Fatty acids; Lipid hydroperoxides; Aldehydes; Hydroperoxides

Contents

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1. Introduction

Polyunsaturated fatty acids (PUFAs) are characterized by presence of one or more -CH=CH-CH_2-CH=CH- groups. They are easily transformed to hydroperoxides (LOOHs) either by enzymes [1–5] or free radicals [6–9]. Such processes are observed after any type of plant injury, either mechanically [10] or by invasion of pests or microorganisms [11]. LPO generation is also observed in germinating plants [12]. In addition, LPO is induced by inflammatory processes [13,14] and aging [15] in man.

LOOHs are unstable. One important degradation process, initiated by bivalent metal ions [16,17], causes cleavage of the peroxy bond to alkoxy-radicals.

$$\text{L-O-O-H} + Fe^{2+} \rightarrow LO^{\cdot} + OH^{\ominus} + Fe^{3+}$$

The $LO^{\cdot}$ radicals are either stabilized by cleavage of an adjacent C–C bond or by reaction of the radical site with the adjacent double bond to form a new radical which adds another molecule of oxygen, generating finally an epoxyhydroperoxide. The latter may be cleaved to an epoxy aldehyde (Fig. 1).

α,β-Unsaturated aldehydes are prone to Michael-type reactions with nucleophiles [19–21]; nucleophiles react also with epoxides by ring opening. α,β-Unsaturated aldehydes and epoxides are toxic [22–24].

Epoxides suffer hydrolysis to corresponding dihydroxy aldehydes. 2,4-alkadienals (see Fig. 1) are converted in aqueous solution slowly to 4-hydroxy-2-alkenals [25] by loss of formic acid in the course of a Baeyer Villiger reaction [26]. The resulting 4-hydroxy-2-alkenals, containing an α,β-unsaturated carbonyl system, undergo, as pointed out above, readily Michael addition and are of high toxicity [24,27,28] (Fig. 2).

Radicals seem to be able to remove a hydrogen from a carbon adjacent to the double bond system. Thus generated mesomeric radicals may add oxygen to form new hydroperoxides. α-Hydroxyaldehydes and 4-hydroxyalkenals are generated by cleavage after reduction of one of the hydroperoxy groups to a corresponding alcoholic group (Fig. 3). Since reduction equivalents are readily available in biological media, reduction of one hydroperoxyl group may occur in an earlier stage as indicated in Fig. 3.

All these aldehydic compounds are detectable after oxidation of pure PUFAs. Their presence in samples of biological origin was so far mainly observed by food chemists, who investigated deterioration of food [29–34]; a few aldehydic compounds were also detected in mammalian tissue samples [24,35–37].

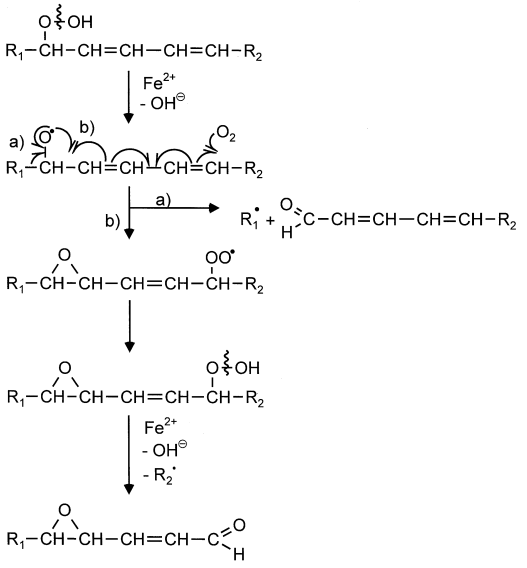


Fig. 1. Generation of aldehydes and 4,5-epoxy-2-enals by cleavage of peroxy bonds in LOOHs, see also the review articles of Frankel [8,18] and Gardner [5,6].

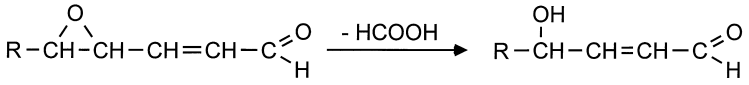


Fig. 2. Transformation of epoxyaldehydes derived from 2, 4-alkadienals to 4-hydroxy-2-alkenals and formic acid.

Since aldehydes represent only trace compounds in tissue, their detection requires chromatographic enrichment and appropriate derivatization for analysis by gas chromatography–mass spectrometry (GC–MS).

The aim of this review is to discuss advantages and disadvantages of different derivatization methods for aldehydic compounds generated by LPO processes in biological materials in combination with their identification by measuring GC retention indices and electron impact (EI) mass spectra (MS).

Aldehydes generated by LPO are of extremely different volatility: Some aldehydes of low mass, e.g. acetaldehyde (b.p. 20.8°C) and propanal (b.p. 48.8°C) are of so high volatility that they are lost partly or completely by manipulation or extraction procedures. Even aldehydes with boiling points above 100°C (e.g. crotonaldehyde, b.p. 104°C) are lost, if the organic solvent used for extraction is removed in vacuum [38].

Such volatile aldehydes are trapped by head space analysis [39]. Solid-phase microextraction (SPME) proved especially efficient. It uses a fine bore fused silica fiber coated with a liquid (e.g. polydi-

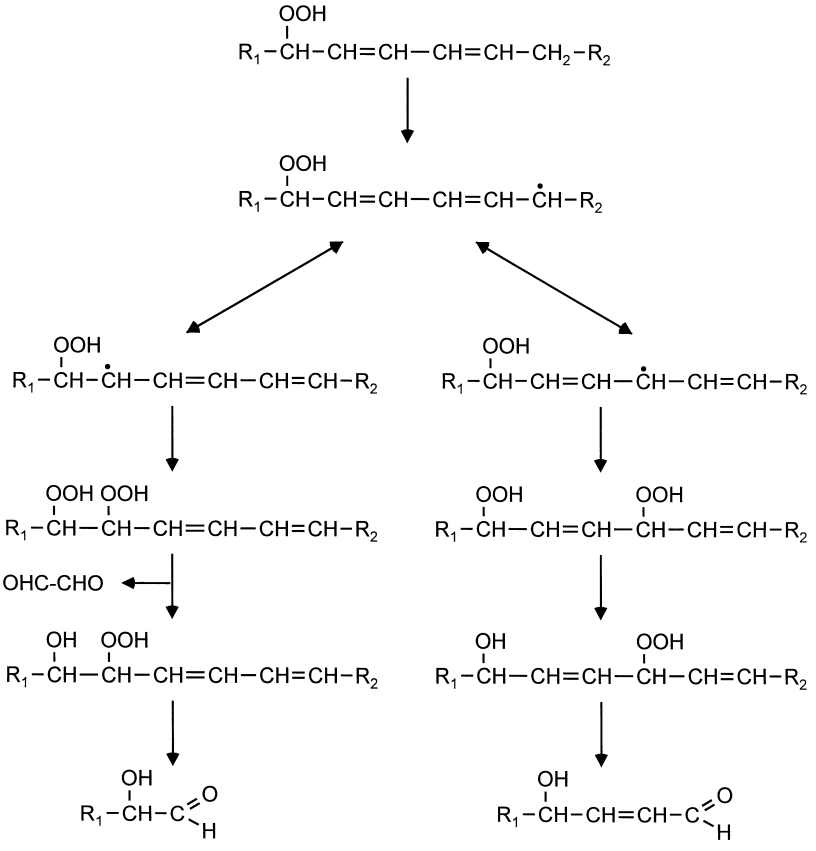


Fig. 3. Suggested mechanism for generation of α-hydroxyaldehydes, 4-hydroxyalkenals and glyoxal by a second dioxygenation reaction, see also Loidl-Stahlhofen [97].

methylsiloxane): The analyte is adsorbed on the fiber and directly introduced in the injector of the gas chromatograph for desorption [40–42]. Otherwise an aldehyde trapping reagent is added to the biological material before any manipulation is done, in order to transform volatile compounds to less volatile ones.

On the other hand, aldehydes with an additional carboxylic function are of so low volatility that they are unable to pass a GC column, requiring their transformation to volatile derivatives. As a consequence, different derivatization methods should be applied for investigation of one and the same sample, and several GC–MS runs are required to elucidate the complete spectrum of aldehydic compounds formed in LPO processes.

One important characteristic of a compound is its molecular mass, which should be deducible from its mass spectrum. Usually soft ionisation methods, or those which generate negatively charged ions, produce abundant molecular ions. Unfortunately these spectra often lack fragment ions, necessary to obtain structural information. Therefore this article is restricted to electron impact mass spectra of appropriate derivatives, which provide in most cases information about the molecular mass and give structural information as well.

Some derivatives, perfectly suited for GC separation, turned out to be of limited value for identification by MS due to absence of molecular ions, others show molecular ions, but lack characteristic fragments. As a consequence, preparation of two different derivatives is sometimes required to obtain structural information. This will be demonstrated by presenting the EI mass spectra of different derivatives of one and the same aldehydic compound.

The most abundant PUFA in biological material is linoleic acid. Hence the review is concentrated mainly to spectra of aldehydic derivatives obtained from linoleic acid. Analogous aldehydes are formed from linolenic or arachidonic acid and higher PUFAs. These spectra are characterized by appropriate shifts of key ions. Thus the discussion of spectra is restricted to only one or two representatives of one compound class. The fragmentation behaviour of homologs is similar in most cases.

A great number of mass spectra of simple aldehydes is recorded in spectra catalogues (e.g. [43] Eight Peak Index References or [44] Stenhagen/McLafferty). Additional references on mass spectra data of aldehyde derivatives are collected in form of an appendix at the end of this review paper.

2. Artificial generation of LPO aldehydes

Cell injury activates enzymes which liberate PUFAs. These are then attacked by lipoxygenases which produce LOOHs. Thus in any homogenization process and by decomposition of LOOHs aldehydes are generated. This fact is impressively demonstrated by daily experience: Strawberries and other fruits do not have odor as long as they are not injured: If the fruit is squeezed by harvesting immediately a typical odor is developed. The only way to restrict this process is destruction of enzymes before cell damage, either by homogenization of biologic material after boiling [45–48], or by addition of an organic solvent [49], but even then enzyme activity is not depressed instantly, thus by extraction with pure methanol generation of LOOHs was recognized [50]. Also if a biological sample is heated up slowly the enzymes start working before they are completely deactivated. The best method for restricting LOOH generation and aldehyde formation by manipulation is inactivation of enzymes by dipping the material to be extracted before cutting into a boiling solvent [47].

3. Derivatization

Aldehydic compounds react in biological surrounding with components containing basic amino groups to Schiff bases, e.g. with lysyl residues of proteins [39,51,52]. Thus only a part of aldehydic compounds is extractable from biological material. The equilibrium between Schiff bases and aldehydes is shifted by addition of aldehydic trapping reagents in favour of reaction with the reagent [37,51,52].

Originally aldehyde trapping reagents were used for characterization of organic compounds: One most important characteristic of an organic compound is its melting point, but most aliphatic aldehydes are liquids. Therefore the liquids were reacted with reagents to transform them into derivatives with a sharp melting point in the range between 100°C and

200°C. Aldehydic derivatives fulfilling this requirements are described in all text books of organic chemistry. They encompass oximes, semicarbazones and hydrazones (e.g. dimethylhydrazones or methoxycarbonylhydrazones and especially phenylhydrazones, in most cases with substituents in the aromatic ring to increase the melting point). Many of these derivatives are not well suited for gas chromatographic separation, since polar groups (e.g. =N-OH groups in oximes and =N-NH-CO-NH_2 groups in semicarbazones) facilitate adsorption and decomposition on GC columns. Addition of inorganic acids is recommended for preparation of some derivatives (e.g. 2,4-dinitrophenylhydrazones): Inorganic acids catalyze isomerization of double bonds and induce hydrolysis of liquid phases in gas chromatographic columns, requiring their perfect removal before a GC analysis, thus complicating the processing.

Therefore special derivatives for trapping aldehydic compounds have been developed in the past. Nevertheless some of the derivatives applied for characterization proved also practicable for GC separation: The most widely used reagent for derivatization of low-molecular-mass aldehydes is 2,4-dinitrophenylhydrazine [53]. The reagent is applied in a solution of 1 M HCl [27] or by addition of perchloric acid [54]. The reaction is carried out at room temperature and is terminated after 2 h. The typical absorption band between 360–380 nm facilitates detection of 2,4-dinitrophenylhydrazones (DNPH) even in trace amounts by HPLC [55,56]; sometimes after a preceding preseparation by TLC [57]. In some cases the components were identified by soft ionization methods after collection of samples [58].

2,4-Dinitrophenylhydrazones are of so low volatility that even formaldehyde is trapped. Formaldehyde and acetaldehyde are present in traces in many solvents. Consequently their detection does not prove that these compounds are generated in the course of LPO processes [27].

Before GC separation, traces of inorganic acids should be removed to avoid deterioration of the column. The reaction of aldehydes with 2,4-dinitrophenylhydrazine generates syn and anti products which are often separated by GC. Thus the aldehyde derivatives are recognized by twin peaks (equally the gas chromatograms of all other hydrazones and oxime derivatives are characterized by such twin peaks). Both isomers give nearly identical mass spectra.

Due to low volatility, 2,4-dinitrophenylhydrazones are only useful for GC separation of low-molecular-mass aldehydes [59], which are separated on DB-1 columns according to their molecular mass and identified for instance with chemical ionization mass spectrometry by registration of positive and negative ions. The chromatograms show often broad peaks, obviously due to partial decomposition. Similar difficulties caused by peak broadening and decomposition of high-molecular-mass compounds in the injector system of the GC are encountered with 4-nitrophenylhydrazones.

Thus 2,4- and 4-nitrophenylhydrazone preparation is only recommended for derivatization of unsubstituted aldehydes. An advantage of this method is avoidance of side reactions (see later), which are encountered with other reagents, e.g. treatment of samples with N-methyl-N-trimethylsilyl-trifluoroacetamide.

A well suited reagent for GC separations is dimethylhydrazine. Dimethylhydrazones are prepared by dissolving the aldehyde containing residue obtained by extraction of biologic material in anhydrous N,N-dimethylhydrazine. Complete reaction requires 1–2 h at room temperature [60]. Dimethylhydrazones possess excellent properties for GC separation, but their preparation requires some precaution due to toxicity of dimethylhydrazine. Further, dimethylhydrazine seems to react incompletely, if the aldehydic material is only present in traces.

Oximes are easily adsorbed on GC column material. Therefore the OH function in oximes is replaced by an organic residue. So for instance methoximes are generated [61–63] when methylhydroxylamine hydrochloride is added in Tris buffer (2-amino-2-hydroxymethyl-1,3-propanediol) to a mixture of aldehydes and when the solution is kept overnight at room temperature. Then the methoximes are extracted with ethyl acetate. Methoximes are less volatile than the corresponding aldehydes but too volatile to allow trapping of propanal and other aldehydes of low mass, if column temperatures are not adjusted for that purpose.

Instead of methoximes, benzyloximes (BO) might be prepared. Benzyloximes are of less volatility than the methoximes [64,65]; thus their application allows identification also of aldehydes of low molecular mass.

Very useful for GC separation are pentafluorobenzyloximes (PFBO) [37,66–68]. Pentafluorobenzyloximes are prepared similarly to methoximes but the reaction is much faster: Aldehydes react quantitatively at room temperature within 30 min [69]; even if aldehydes are in aqueous solution [70] or bound to proteins in the form of Schiff bases [71]. The presence of fluorine atoms in the molecule allows improvement in detection limits by applying electron capture detectors.

The reaction of a reagent with an aldehyde is highly dependent on the localization of a positive partial charge at the carbonyl carbon. If this charge is distributed by resonance, e.g. in 2-alkenals, the reaction rates are decreased considerably. This should be always kept in mind. Different reagents react also with different rate: the reaction with pentafluorobenzylhydroxylamine is much faster than that with O-methylhydroxylamine, obviously depending on the electron density at the nitrogen.

The presence of polar functional groups in an aldehyde requires additional derivatization. Thus carboxylic groups must be blocked by esterification. In most cases diazomethane is used for this purpose. COOH groups of pure samples react with an etheral diazomethane solution within seconds [50]; but, according to own observations, completion of the reaction requires about 2 min, if samples were obtained from biological sources. Excess reagent is blown off in a stream of nitrogen. If the reaction time is extended for more than a few minutes, α,β-unsaturated carbonyl compounds may react in appreciable amounts to Δ^1-pyrazolines [72,73], which decompose by the following gas chromatographic separation by loss of N_2, thus forming methylderivatives of the original compound and other products [72,73]. Addition of diazomethane to conjugated double bonds is avoided, if the carbonyl group is first transformed in a derivative (e.g. a methoxime) and methylation is carried out in the following step [74]. In addition diazomethane may react with aldehydes and cyclic carbonyl compounds by methylen insertion [75].

On the other hand, protection of the carboxylic groups is achieved by trimethylsilylation (for an excellent review see van Look et al. 1995) [76]. COOH groups are readily transformed to trimethylsilylesters (within minutes) by reaction with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) or another silylating reagent, e.g. hexamethyldisilazane-trimethylchlorosilane-pyridine [5] but care must be taken to avoid hydrolysis: Even by storing in an atmosphere of air or by chromatography of trimethylsilylesters more or less complete hydrolysis to the acids is observed [77], this is combined with a partial or complete loss by adsorption on GC columns. When samples are introduced in the GC with an excess of MSTFA, hydrolysis is avoided.

The reagent transforms also OH or NH_2 groups to trimethylsilylderivatives [77], NH_2 groups usually react by exchange of both hydrogens. The N-bis-trimethylsilylated compounds are more sensitive than trimethylsilyl esters to traces of water, thus even by manipulation partial hydrolysis is observed, resulting in generation of the mono- and bis-trimethylsilylated amine derivatives side by side.

In addition, MSTFA reacts with aldehydic groups by adduct formation [78]. The adducts are volatile, their EI mass spectra are rather informative. The adduct formation is also highly dependent on the carbonyl activity: α,β-unsaturated aldehydes react much slower than saturated ones due to a lower electron density at the carbonyl carbon. In many cases adducts are detected together with free aldehydes. The yield of adduct increases with time, therefore to avoid adduct generation samples should be investigated by GC–MS not longer than 1 h after addition of MSTFA.

Less sensitive to hydrolysis compared to trimethylsilylderivatives are *tert*.-butyldimethylsilyl derivatives of alcoholic groups. These derivatives are used occasionally for derivatization of hydroxy groups [79–82].

Some aldehydic compounds contain hydroxy groups. Aldehydes with a hydroxy group in position 2 suffer easily dehydration to α,β-unsaturated aldehydes. Thus, for instance, 2-hydroxyheptanal decomposes to 2-heptenal, often reported as flavour constituent [30,83–85]; but in fact, 2-heptenal is an artifact. Similarly 2,4-dienals are generated by dehydration of 4-hydroxyalkenals, e.g. 2,4-nonadienal

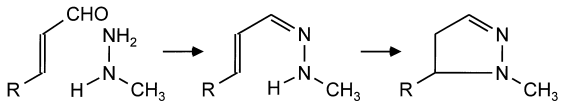


Fig. 4. Reaction of 2-alkenals with methylhydrazine according to Tamura [88].

[30,83,85,86] is an artifact of 4-hydroxy-2-nonenal. Decomposition of these and other hydroxyaldehydes is avoided if MSTFA is added before GC analysis [87].

A special reagent was developed for derivatization of 2-alkenals: methylhydrazine [88]. The primarily generated methylhydrazone reacts further by Michael addition to a N-methylpyrazoline (NMP) (Fig. 4).

Best yields are obtained by BF_3 catalysis [89,90]. Analysis of the latter with a nitrogen GC detector allows recognition of the adducts with high sensitivity. A drawback of the method seems to be the poor stability of methylpyrazolines and the low volatility of high molecular mass representatives of this class of compounds [90].

4. Experimental

Gas chromatography was carried out with a Carlo Erba Instruments HRGC 5160 Mega Series gas chromatograph equipped with a flame ionisation detector using a DB-1 fused silica gel capillary column (30 m×0.32 mm I.D.) (J & W Scientific, Germany), covered with a 0.1 μm layer of liquid phase. The temperature of the detector was kept at 290°C, the injector temperature was 280°C. Injector volume: 0.2–0.7 μl of a 1% (m/v) solution. Temperature program: 5 min isotherm 60°C, 3°C/min 60°C to 280°C, then 15 min isotherm at 280°C. The carrier gas was hydrogen and the splitting ratio 1:30. Peak integration and data recording was done with a Merck Hitachi Chromatointegrator D-2500.

GC–MS was performed on a Finnigan MAT 95 double focussing mass spectrometer with an inverse Nier-Johnson geometry, equipped with an EI ion source operated at 70 eV. A Hewlett Packard 5890 Series II gas chromatograph with a fused silica DB-5 glass capillary column (30 m×0.25 mm I.D., covered with a layer of 0.1 μm of liquid phase; J and W Scientific, Germany) was used for sample separation. The injector temperature was kept at 280°C, injection volumes were 0.6–1.5 μl of a 1%–2% (m/v) solution. Temperature program: 3 min isotherm 50°C, within 2 min increase to 100°C, then 3°C/min till 300°C, finally 10 min isotherm at 300°C.

Kováts indices were determined by coinjection of a 0.2 μl sample of a standard mixture of saturated straight chain alkanes (C_{10}–C_{36}) [91].

Spectra were run from synthetically prepared samples which were purified by TLC. Only a few hydroxy compounds were not synthetically available. Spectra of these compounds were obtained by LPO of linoleic acid and separation by GC–MS. These spectra are indicated by an asterisk. It might be that these spectra contain low amounts of impurities.

Retention index (I)[1] [92] of synthetic samples were determined by GC with a DB-1 liquid phase, while I-values of GC–MS runs were determined on a DB-5 liquid phase. The values on the DB-1 and DB-5 column are slightly different, retention indexes obtained on a DB-5 column are marked also by an asterisk.

Propanal, butanal, pentanal, hexanal, trans,trans-2,4-decadienal, malondialdehyde-tetrabutylammonium salt, glyoxal and glyoxylic acid monohydrate, were commercially available from Fluka, Buchs, Switzerland, trans-2-octenal was purchased from Sigma-Aldrich GmbH, Steinheim, Germany and trans,trans-2,4-nonadienal from Acros Organics (Fisher Scientific, Ingolstadt, Germany).

4,5-Epoxy-2-decenal was available by epoxidation of trans,trans-2,4-decadienal by treatment with Adams reagent (dimethyldioxirane) [93]: Briefly 1 ml of a solution of dimethyldioxirane in acetone was added to 1–2 mg of trans,trans-2,4-decadienal at 4°C. After standing over night the reagent was blown off by a stream of nitrogen. Dimethyldioxirane exclusively adds at the 4,5 and never at the 2,3 double bond.

4-Hydroxy-2-nonenal was available from 3-nonen-1-ol (Fluka, Buchs, Switzerland) by treatment with

[1] $I = 100 \cdot (y - x) \cdot \{[\log(t_{R(A)}) / \log(t_{R(x)})] / [\log(t_{R(y)}) / \log(t_{R(x)})]\} + 100 \cdot x$: x and y: Number of carbon atoms of n-alkanes eluting before and after the substance A; $t_{R(A)}$ = retention time of substance A; $t_{R(x)}$ = retention time of alkane eluting before A; $t_{R(y)}$ = retention time of alkane eluting after A.

m-chloroperbenzoic acid [94] followed by oxidation with pyridinium chlorochromate (PCC) in analogy to a procedure developed by Piancatelli [95]: 710 mg (5.0 mmol) 3-nonen-1-ol and 862.5 mg (5.0 mmol) *m*-chloroperbenzoic acid (MCPBA) were dissolved in 30 ml CH_2Cl_2. After 3h at room temperature the generated MCBA was removed by extraction with aqueous $NaHCO_3$ solution. CH_2Cl_2 was then evaporated at 30°C using a rotation evaporator. The residue, 3,4-epoxy-nonan-1-ol, was subjected to oxidation with pyridinium chlorochromate: 650 mg (3.0 mmol) PCC were suspended in 10 ml dry CH_2Cl_2. 320 mg (2.0 mmol) of 3,4-epoxy-nonan-1-ol, dissolved in 5 ml dry CH_2Cl_2, were added rapidly at room temperature. After standing for 90 min at room temperature 10 ml dry diethylether were added, the solution was filtered and the solvent (diethylether) removed with a rotation evaporator. The residue was dissolved in a mixture of cyclohexane (CH)–ethylacetate (EA) (3:1) and purified by TLC ($R_F = 0.27$).

2-Hydroxyheptanal was obtained from heptanol following a procedure reported by Barbier and Benezra [96]: Heptanal was treated with acetic anhydride and K_2CO_3 at 150°C for 24 h, in order to prepare its enol acetate. This was epoxidized using MCPBA. The obtained 1,2-epoxy-1-acetoxyheptane was transformed into 2-acetoxy-heptanal by treatment with *p*-toluenesulfonic acid. 2-hydroxy-heptanal was generated from the latter by treatment with hog liver esterase (Fluka Chemie AG, Buchs, Switzerland): Briefly 13.1 mg 2-acetoxy-heptanal were dissolved in 100 ml phosphate buffer (pH = 7). 563 u of hog liver esterase were added. The solution was kept at room temperature for 24 h. The liberated 2-hydroxy-heptanal was extracted with diethylether. This extract was used for derivatization, since removal of solvent generates dimers and polymers – we failed to isolate 2-hydroxy-heptanal free of solvent.

The preparation of 6-hydroxy-2,4-undecadienal is reported elsewhere [97].

4,5-Dihydroxy-2-decenal was generated from 4,5-epoxy-2-decenal (see above): 4.6 mg 4,5-epoxy-2-decenal were treated in an atmosphere of N_2 at room temperature with 1 ml of 5% HCl. After 5 min the solution was neutralized with an aqueous $NaHCO_3$ solution. The product was extracted with ethyl acetate.

Methyl-9-oxononanoate was obtained by $NaIO_4$ cleavage of aleuritic acid (9,10,16-trihydroxy-hexadecanoic acid) according to Reuter and Salomon [98]. The obtained products methyl-9-oxononanoate and 7-hydroxy-heptanal were separated by TLC (CH-EA, 3:1), $R_F = 0.43$.

Methyl-8-oxooctanoic acid was prepared according to Brown and Subba Rao [99] by reacting methyl-8-oxo-8-chlorooctanoate (Sigma-Aldrich Chemie) with lithium tri-*tert*.-butoxyaluminohydride (Sigma-Aldrich Chemie).

The *bis*-trimethylsilylacetal of methyl-9-oxononanoate was obtained as by-product by treatment of methyl-9-oxononanoate with MSTFA: 10 μl of MSTFA were added to 1–2 mg of methyl-9-oxononanoate. After standing overnight at room temperature the reaction product was directly injected into the GC–MS system.

Methyl-11-oxo-9-undecenoate was synthesized by a Wittig reaction starting from methyl-9-oxononanoate in analogy to a procedure reported by Bestmann [100]: 0.91 g of methyl-9-oxononanoate were dissolved in 300 ml benzene. 1.6 g triphenylphosphoranylidene-acetaldehyde ($Ph_3P=CH$-CHO) obtained from Acros Organics, (Ingolstadt, Germany) were added. The solution was refluxed overnight under N_2. After removal of the solvent the residue was extracted with cold diethylether. The raw product obtained by evaporation of the solvent was purified on silica gel by chromatography with a mixture of CH-EA (3:1).

The enol-trimethylsilylether of methyl-11-oxo-9-undecenoate was obtained by treatment of 1 mg methyl-11-oxo-9-undecenoate in a reacti-vial with 10 μl MSTFA by addition of one grain K_2CO_3 and heating to 50°C for 24 h.

Methyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate was generated enzymatically: 250 μl of linoleic acid were suspended in a 50 ml solution of 200 mM borate buffer (pH 9) and subjected to ultrasonification. 500 mg lipoxygenase from soybean (Fluka) were added. The solution was stirred for 30 min at room temperature. Then a pH of 4 was adjusted by addition of 0.5 molar HCl. The solution was extracted two times with each 150 ml of $CHCl_3$. The organic layers were combined, cylohexane was added in order to remove water traces with the solvent by rotation evaporation. The residue was dissolved in a solution of hexane–

diethyl ether–acetic acid (60:40:1) and separated by TLC. Methyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate was found in the zone R_F 0.32-0.36. Yield 8.25 mg.

The bis-trimethylsilylacetal of methylglyoxalate was obtained as byproduct by reacting methylglyoxylic acid monohydrate with MSTFA.

No synthetic samples were available from methyl-9-hydroxy-10-oxodecanoate, methyl-8-hydroxy-9-oxononanoate, methyl-10-hydroxy-9-oxodecanoate and methyl-9-hydroxy-12-oxo-10-dodecenoate. These compounds were detected after air oxidation of linoleic acid induced by $FeCl_3$ in 0.1 *M* phosphate buffer (pH 7.4) at room temperature as described recently [101]. Oxidation was stopped after 4 days by addition of 10 μl $P(OCH_3)_3$. The solution was treated for 5 min to reduce hydroperoxides. The resulting solution was divided in three equal parts: Part one was used to prepare pentafluorobenzyloximes, part 2 for preparation of methoximes and part 3 for generation of MSTFA adducts.

4.1. Generation of pentafluorobenzyloximes

A 0.05 *M* methanolic pentafluorobenzylhydroxylamine·HCl solution was added in a tenfold molar excess to part 1. The solution was stirred for 60 min at 20°C. The pH was controlled and if necessary adjusted to pH 7.4 by addition of 2 N NaOH. The solution was then extracted three times with each 25 ml chloroform. The combined $CHCl_3$ layers were dried with Na_2SO_4. After filtration the solvent was removed at 20°C using a rotation evaporator. The residue was dissolved in 10 μl ethyl acetate, transferred to a Reacti-Vial, and 200 μl of an etheral diazomethane solution were added. After 1–2 min the excess of diazomethane and solvent were removed with a stream of nitrogen. When shorter reaction times are applied, as sometimes recommended [50], carboxylic groups may not be transformed completely to their methylesters. Then 10 μl of MSTFA were added to the residue obtained after methylation. The mixture was shaken for 60 min at 40°C in a thermomixer. Finally the reaction mixture was diluted with 90 μl of dry ethyl acetate, 0.2–0.3 μl of this solution were injected into the GC–MS instrument.

Part 2 of the reduced oxidation mixture was used to prepare methoxime derivatives: A 0.05 *M* solution of O-methylhydroxylamine hydrochloride (MHA·HCl) in methanol was added in about 50 molar excess to the aqueous oxidation mixture after $P(OCH_3)_3$ reduction. The solution was stirred 24 h at 20°C, by control of the pH: If necessary the pH was adjusted to 3–4 by addition either of 2 M HCl or 2 M NaOH. The reaction mixture was extracted three times with each 25 ml $CHCl_3$ and methylated with diazomethane and trimethylsilylated with MSTFA as described above for preparation of pentafluorobenzyloximes.

Part 3 was treated with diazomethane and MSTFA, without any previous reaction with an aldehyde trapping reagent.

5. Mass spectra

5.1. Saturated straight chain aliphatic aldehydes

Decomposition of hydroperoxides of $n-3$ and $n-6$ fatty acids generates propanal and hexanal, respectively. These aldehydes are of high volatility. Therefore they escape often detection – and especially quantification – except by head space analysis [102] or by sample adsorption on poly(dimethylsiloxane) fibers that can be directly inserted into a GC system [40–42].

Mass spectra of saturated aliphatic aldehydes [103] are not very informative (see Fig. 5). Molecular ions are rarely detectable, peaks are present which correspond to loss of water or CO. A peak at m/z 44 is generated by a McLafferty rearrangement reaction [104].

Spectra of methoximes derived from straight chain saturated aldehydes with 3 and more carbon atoms are characterized by an ion of m/z 73 formed by a McLafferty rearrangement and a fragment 13 u (mass units) heavier, (''McLafferty +13 ion'') of m/z 86 (Fig. 6). The fragment of m/z 86 is generated in a double hydrogen rearrangement reaction as outlined in Fig. 6. Molecular ions usually are only of low abundance (Figs. 7 and 8).

MSTFA reacts with aldehydic groups by adduct formation [78] (Fig. 9). The mass spectra of MSTFA adducts derived from aliphatic aldehydes [108,109] are characterized by key ions of mass 110, 134, 184 and 228. Although molecular ions are often missing,

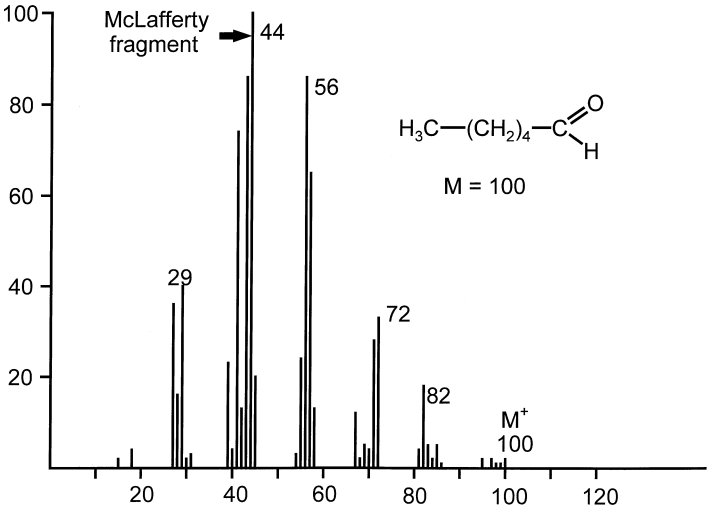


Fig. 5. Mass spectrum of hexanal according to Gilpin [103] ($I=786$).

the molecular mass may be deduced from a M−15 ion.

Even more important for confirmation of the molecular mass are fragments arising by loss of the -N(CH_3)-$COCF_3$ group generating a fragment ion corresponding to M−126 (Figs. 10–12).

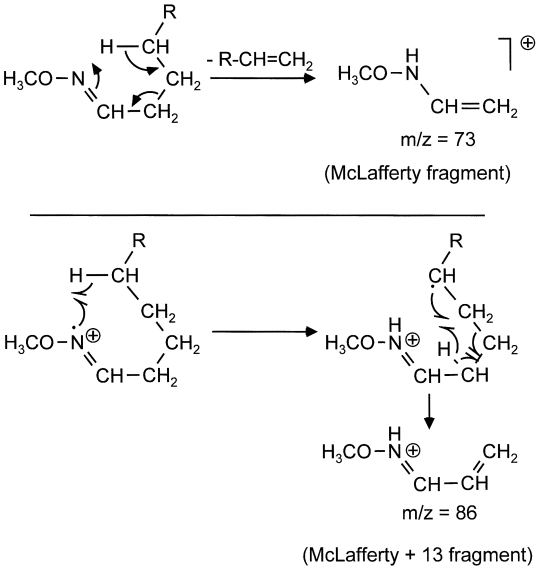


Fig. 6. McLafferty rearrangement generates from methoximes a key ion of mass 73 [105,104], while a double hydrogen rearrangement produces the ''McLafferty+13 ion'' [106,107].

The most dominant peaks correspond to $(CH_3)_3Si^+$ ions (m/z 73), accompanied by ions of mass 75:

$$(H_3C)_2Si=\overset{\oplus}{O}H$$

These ions indicate the presence of a trimethylsilyl group and occur in all spectra of compounds with a TMS group.

A not very prominent, but for elucidation of structurally unknown compounds an important degradation route starts by hydrogen migration from a CH_2 group in the alkyl residue to the ionized carbonyl group. This migration causes then cleavage of an adjacent C–C bond. Thus a series of fragment ion peaks every 14 u apart are recognized in the spectra (Fig. 13):

Aldehydes might also be transformed first to oximes and these can be reacted in a second step with N-*tert*.-butyldimethylsilyl-N-methyltrifluoroacetamide. The mass spectra of the latter are characterized by intense peaks, indicating the loss of the *tert*.-butyl radical at M−57 (Fig. 14).

EI mass spectra of positive ions derived from pentafluorobenzyloximes of aliphatic saturated aldehydes are characterized by the ion of the pentafluorobenzyl ion of m/z 181. Scanning the spectra of a GC run for this ion turned out to be very useful to recognize aldehydic compounds even in complex mixtures [87,109].

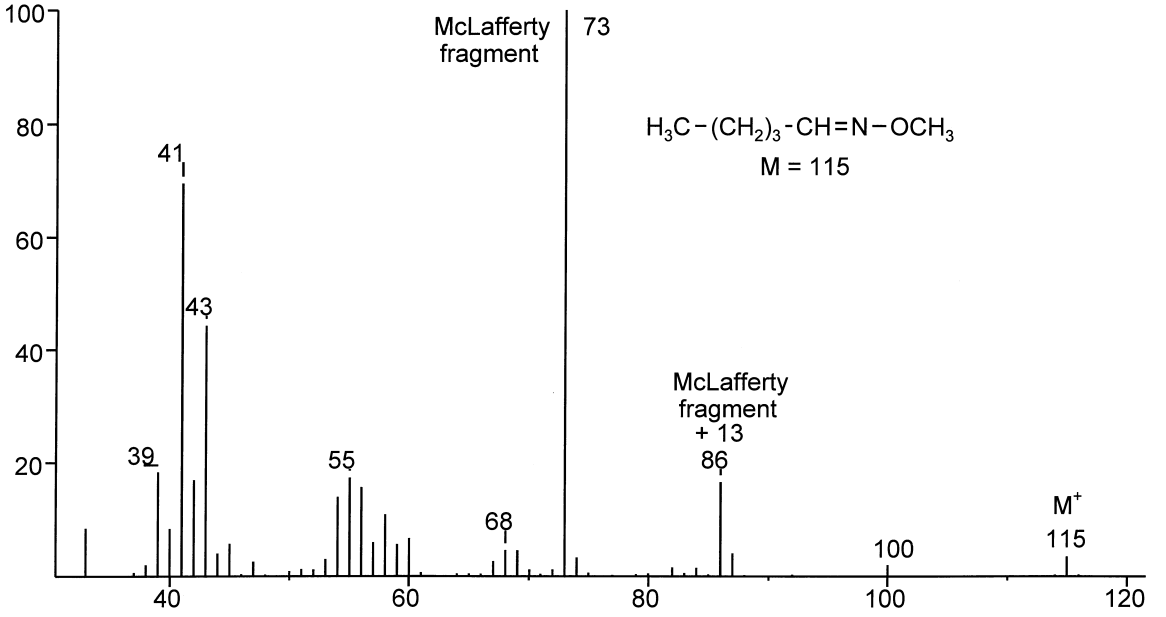


Fig. 7. Mass spectrum of the methoxime of pentanal ($I_1 = 810$; $I_2 = 815$).

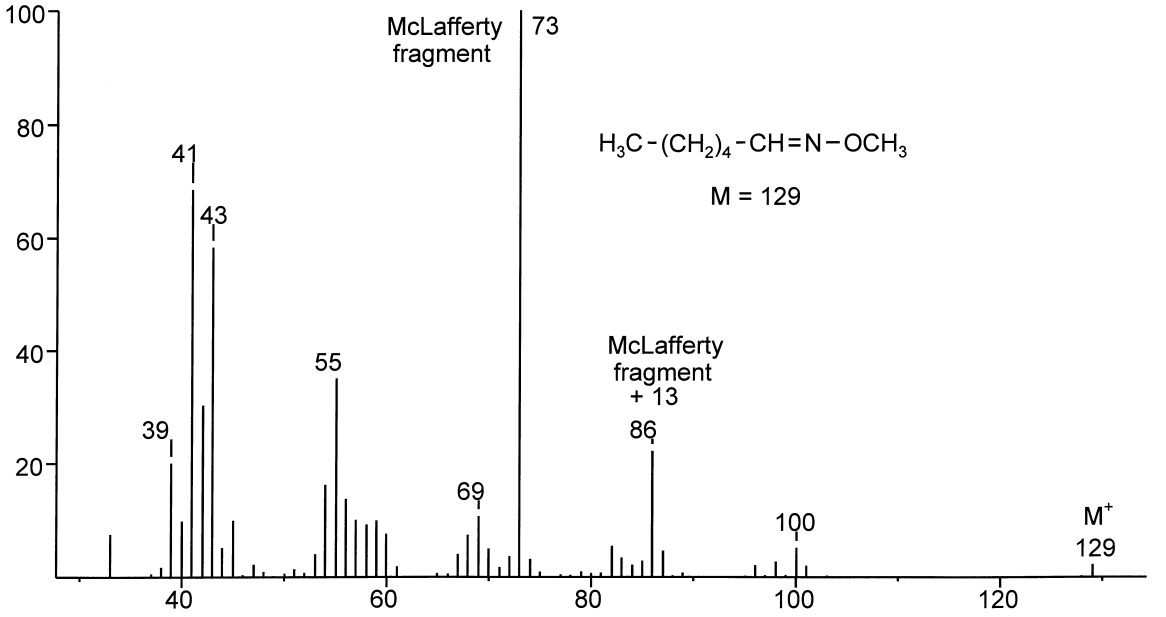


Fig. 8. Mass spectrum of the methoxime of hexanal ($I_1 = 910$; $I_2 = 915$).

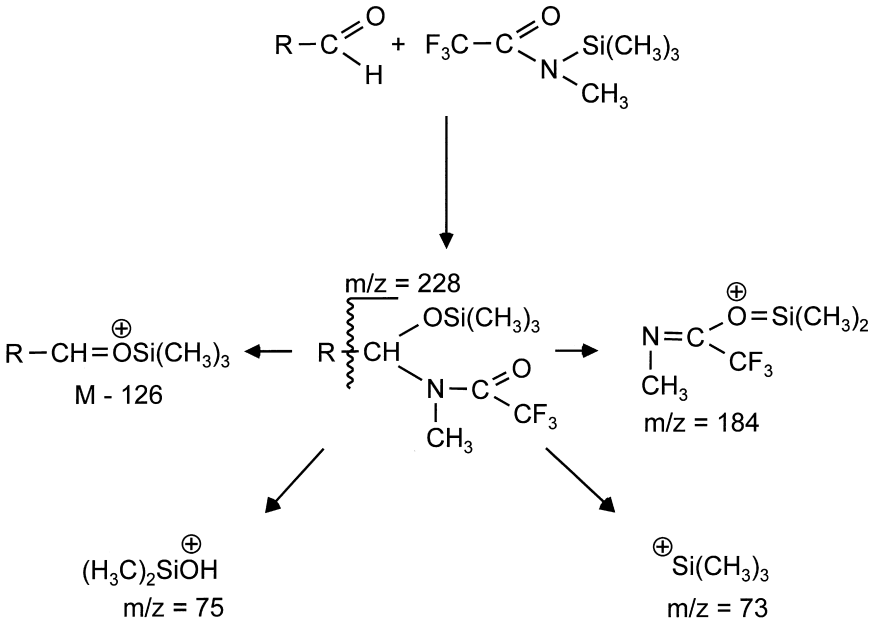


Fig. 9. MSTFA adduct formation of aldehydes and their main fragmentation routes according [78].

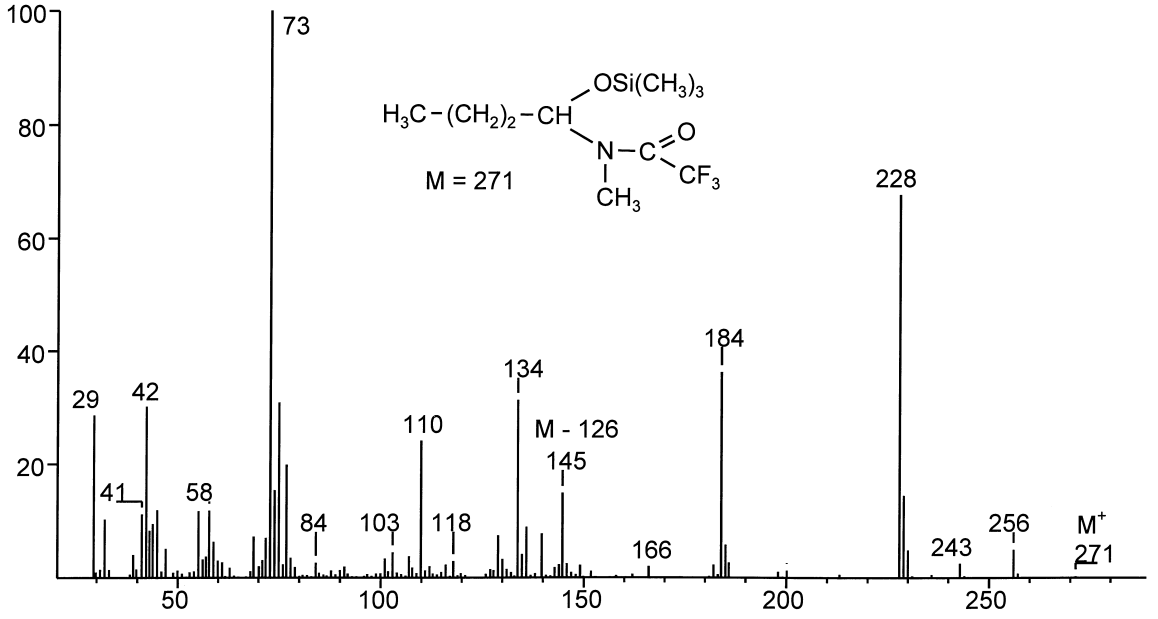


Fig. 10. Mass spectrum of the MSTFA adduct of butanal ($I = 1122$).

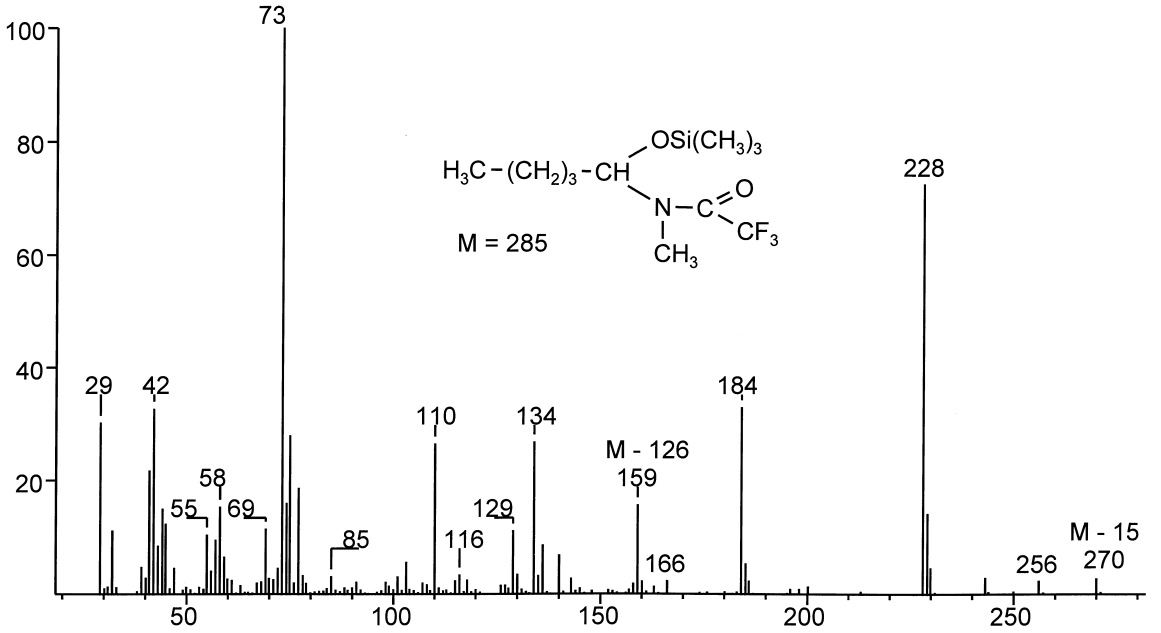


Fig. 11. Mass spectrum of the MSTFA adduct of pentanal ($I = 1208$), see also Luftmann and Ende [78].

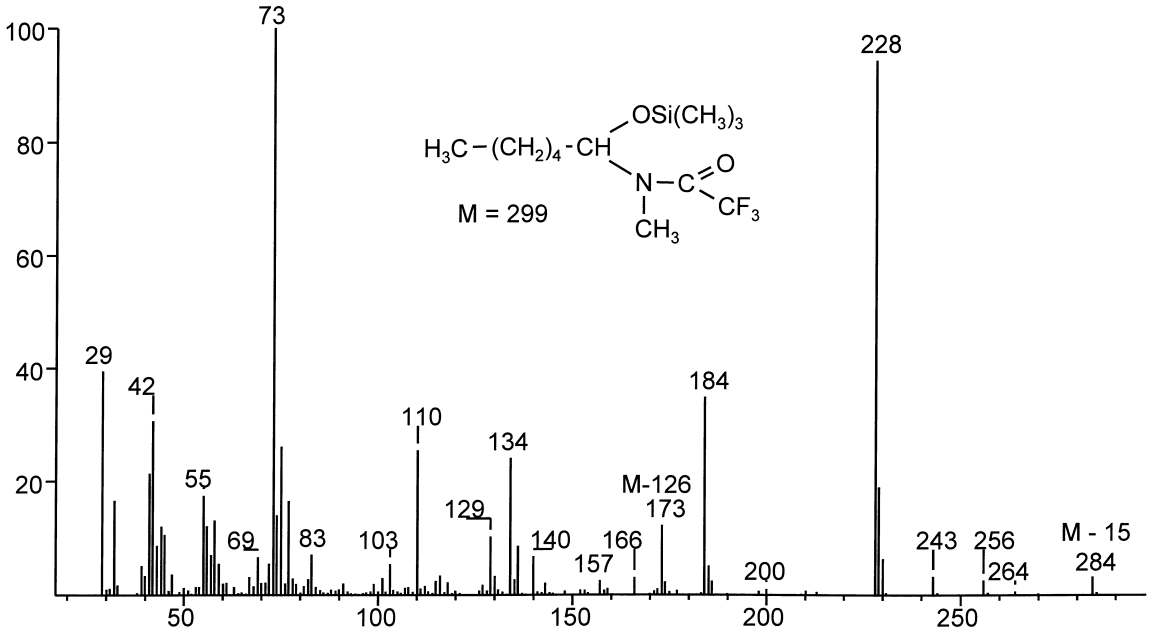


Fig. 12. Mass spectrum of the MSTFA adduct of hexanal ($I = 1297$), see also Luftmann and Ende [78].

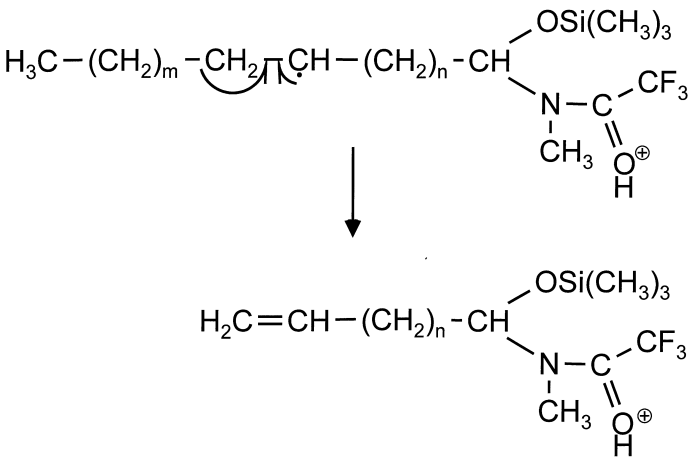


Fig. 13. Hydrogen abstraction by the carbonyl group followed by C-C bond cleavage induces the generation of a series of fragment peaks every 14 u.

Unfortunately molecular ions in the spectra of pentafluorobenzyloxime (PFBO) derivatives are often missing, fragment ions show sometimes only low intensity. Deduction of the molecular mass is therefore difficult, especially in samples of biological origin, which contain often small amounts of impurities. Usually M−17 (M–$OH^{\cdot}$) ions are observed with low intensity (Figs. 15–17). Typical for aliphatic aldehydes with a longer alkyl chain are ions of mass 239, generated by McLafferty rearrangement (Fig. 18):

Mass spectra of 2,4-dinitrophenylhydrazones are characterized by intense molecular ions [110]. While degradation of 2,4-dinitrophenylhydrazones derived from formaldehyde and acetaldehyde in the mass spectrometer is started by loss of $^{\cdot}OH$ and H_2O,

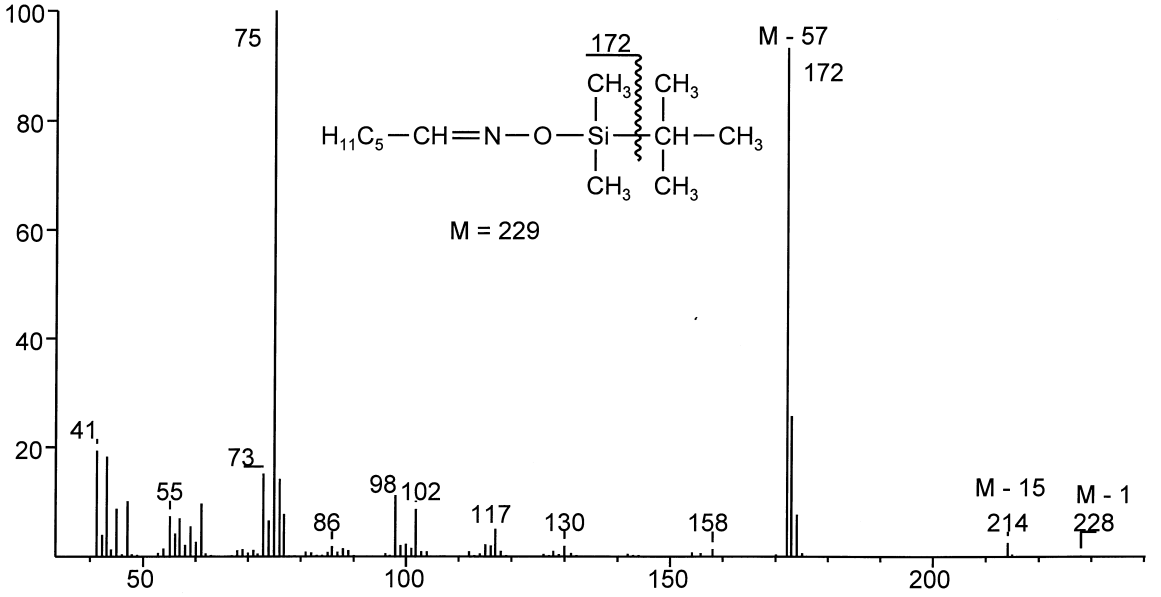


Fig. 14. Mass spectrum of the *tert.*-butyldimethylsilyl-oxime derivative of hexanal ($I_1 = 1290$; $I_2 = 1303$).

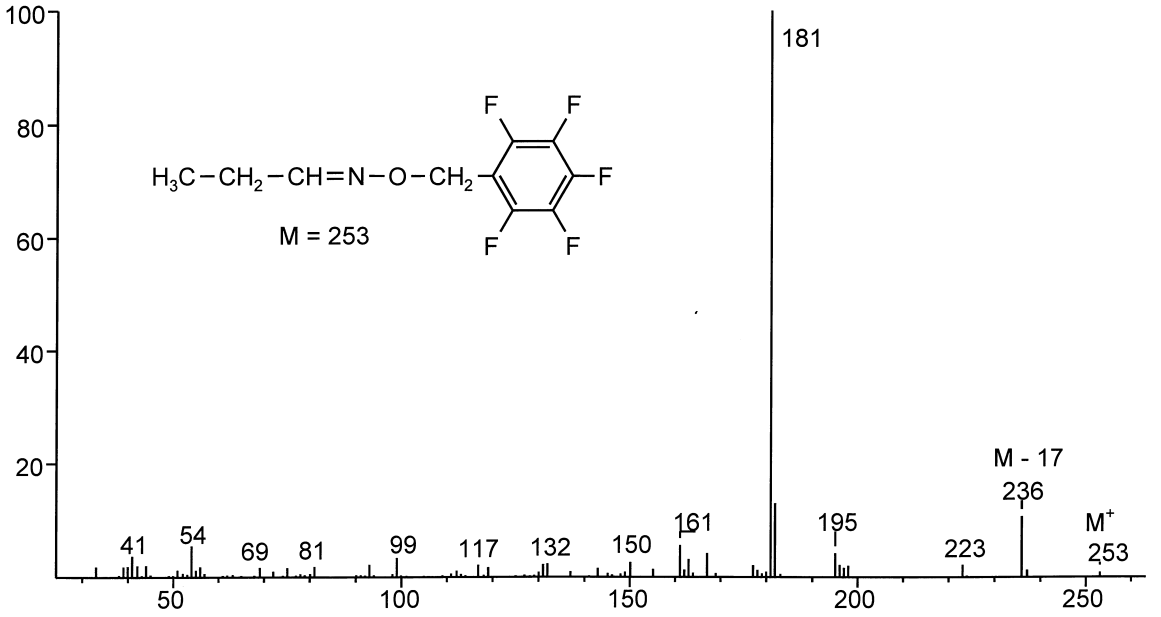


Fig. 15. Mass spectrum of the PFBO derivative of propanal ($I_1 = 1162$; $I_2 = 1169$).

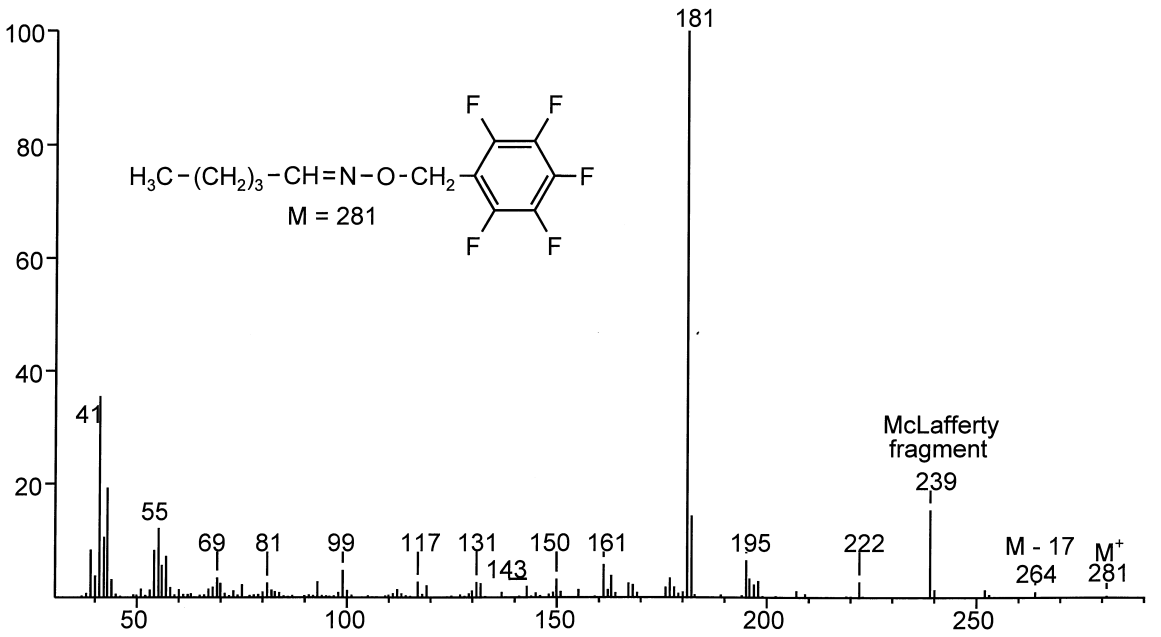


Fig. 16. Mass spectrum of the PFBO derivative of pentanal ($I_1 = 1342$; $I_2 = 1349$).

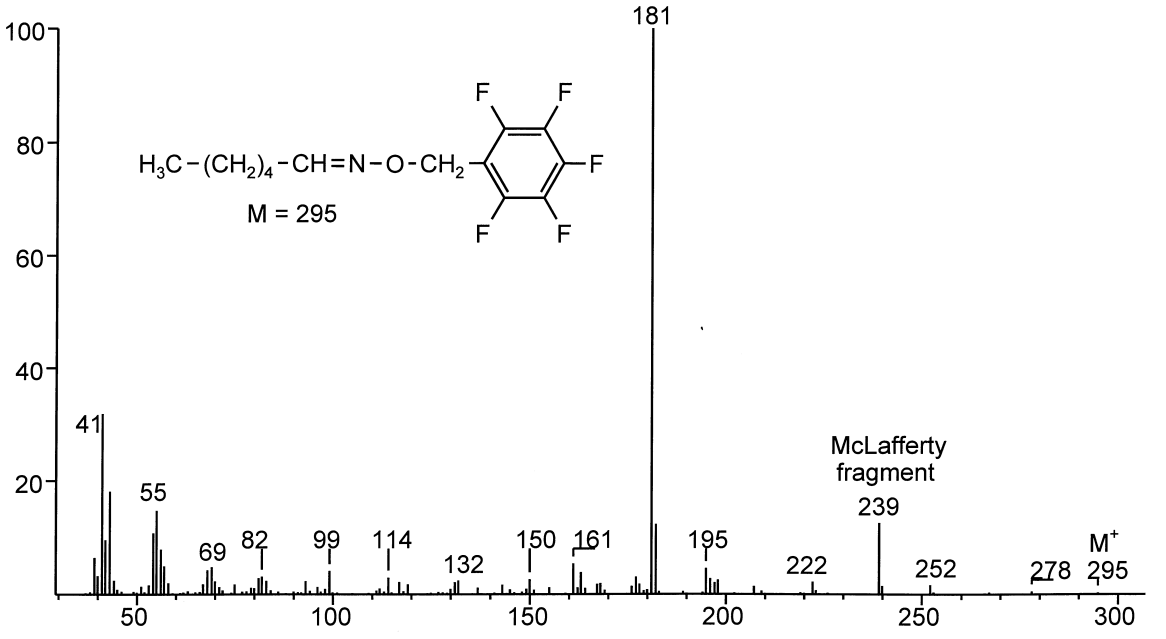


Fig. 17. Mass spectrum of the PFBO derivative of hexanal ($I_1 = 1435$; $I_2 = 1443$).

aldehydes with 5 and more carbon atoms suffer mainly a McLafferty rearrangement, leading to intense ions of mass 224 (Fig. 20). These react further by loss of water to form ions of mass 206 (Fig. 19).

Aliphatic 2,4-dinitrophenylhydrazones of low mo-

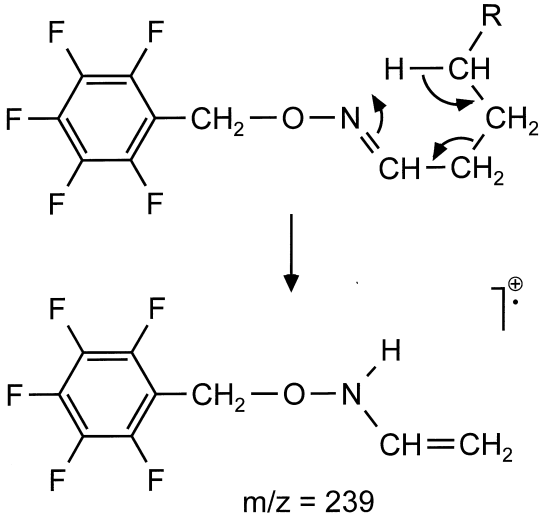


Fig. 18. Generation of the ion m/z 239 in spectra of PFBO derived from saturated aldehydes.

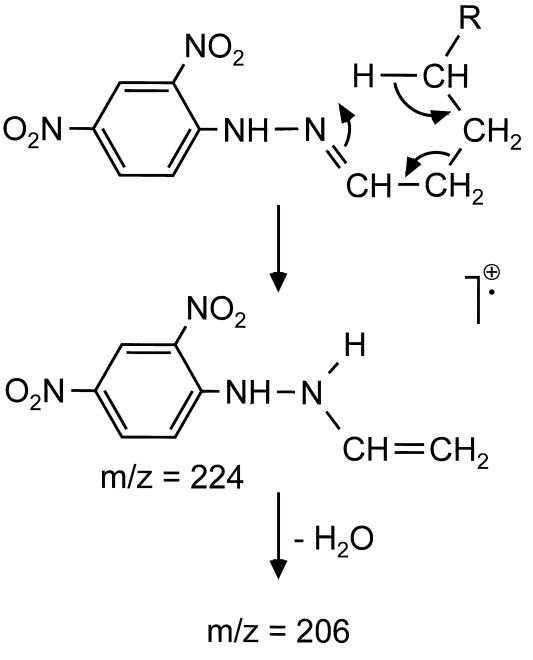


Fig. 19. Generation of the fragments of m/z 224 and 206 from 2,4-dinitrophenylhydrazones of aliphatic aldehydes according to Djerassi [111].

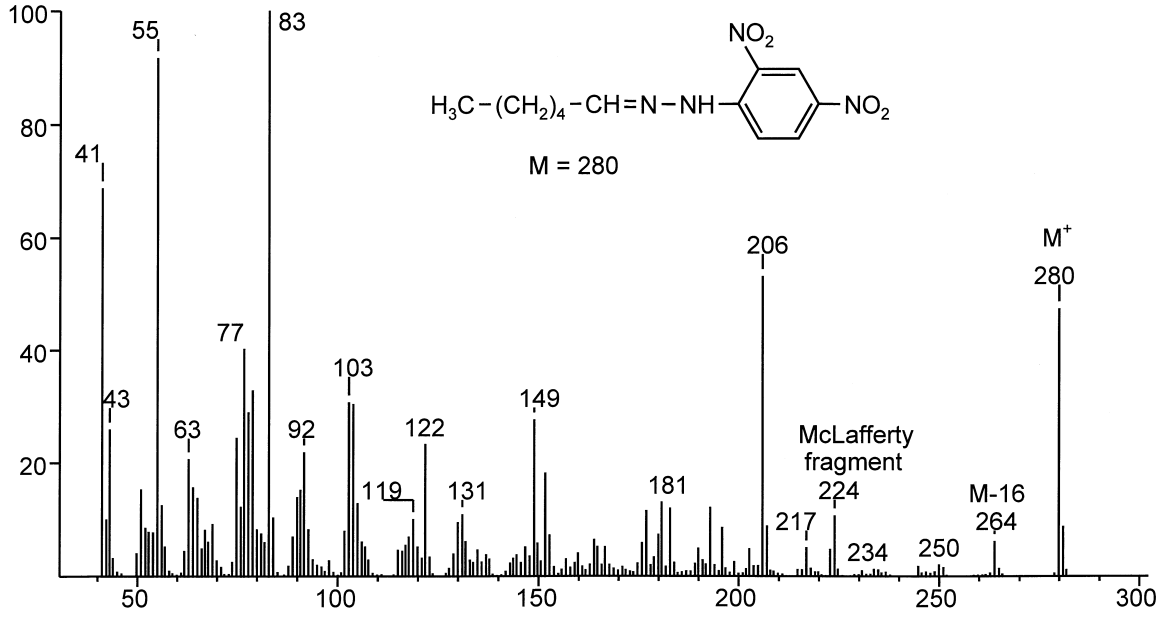


Fig. 20. Mass spectrum of the 2,4-dinitrophenylhydrazone of hexanal ($I_1 = 2362$; $I_2 = 2410$) compare Kleipool [113] and Goldsmith [114].

lecular mass are sufficiently volatile to achieve their separation by GC. Since the mass spectra of 2,4-dinitrophenylhydrazones derived from low molecular mass aldehydic compounds show molecular ions, which are missing in other derivatives, e.g. in pentafluorobenzyloximes, they are better suited for detection of low-molecular-mass saturated aldehydes than corresponding PFBO derivatives, in spite of partial decomposition on GC columns.

In some instances 4-nitrophenylhydrazones were prepared. Mass spectra of 4-nitrophenylhydrazones [112] are characterized like 2,4-dinitrophenylhydrazones by intense molecular ions (Fig. 21). In addition 4-nitrophenylhydrazones are prone to undergo a McLafferty rearrangement. Thus they are more informative than those of 2,4-dinitrophenylhydrazones. Since 4-nitrophenylhydrazones are also of higher volatility than 2,4-dinitrophenylhydrazones this type of derivatives is well suited for GC–MS investigations of aldehydic compounds.

The mass spectra of dimethylhydrazones of saturated aliphatic aldehydes are even more informative than those of 4-nitrophenylhydrazones: They show intense molecular ions [114]. Two main fragments, typical for dimethylhydrazones of saturated aliphatic aldehydes, are ions of mass 44 and 85 (Figs. 25 and 26): The ion of mass 44 is obviously generated by a double hydrogen rearrangement, as outlined in Fig. 22:

Abstraction of hydrogen from a CH_2 group generates a radical, which induces a back shift of a hydrogen from one of the methyl groups followed by generation of the fragment: $H_2C=\overset{\oplus}{N}H-CH_3$.

The ion of mass 85 is obtained by allylic cleavage (Fig. 22), the accompanying ion of mass 86 by a McLafferty rearrangement. The ion of mass 99 corresponds to the ''McLafferty +13 ion''. Such ions are generated by all aldehyde derivatives with a -N=CH group (Fig. 23) and a chain with several CH_2 groups [107].

An ionized -CH=N- group triggers hydrogen abstraction from a nearby located CH_2 group. The thus generated protonated $-H\overset{\oplus}{N}=CH-$ group activates the hydrogens in the adjacent CH_2 group, inducing back shift of a hydrogen and cleavage of the adjacent bond (Fig. 23).

Alternatively the intermediate of the reaction is stabilized by cleavage of the adjacent bond (Fig. 24):

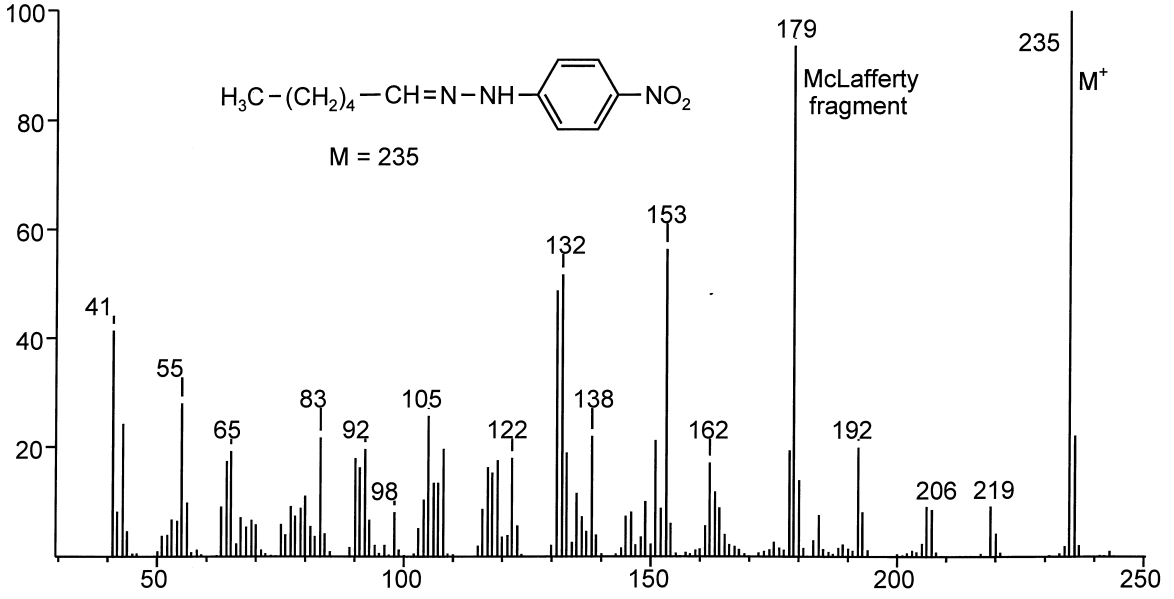


Fig. 21. Mass spectrum of the 4-nitrophenylhydrazone of hexanal ($I = 2259$) compare Goldsmith [114].

Thus mass spectra of dimethylhydrazones of straight chain aldehydes show a series of fragment ion peaks 14 u apart (m/z 99, 113, 127 and so on, (Fig. 26), allowing recognition of the presence or absence of a straight chain aldehyde derivative. These fragments are less abundant in other aldehyde derivatives.

The low volatility of semicarbazones, among the most commonly prepared derivatives of aldehydes, exclude their use for aldehyde analysis by GC–MS.

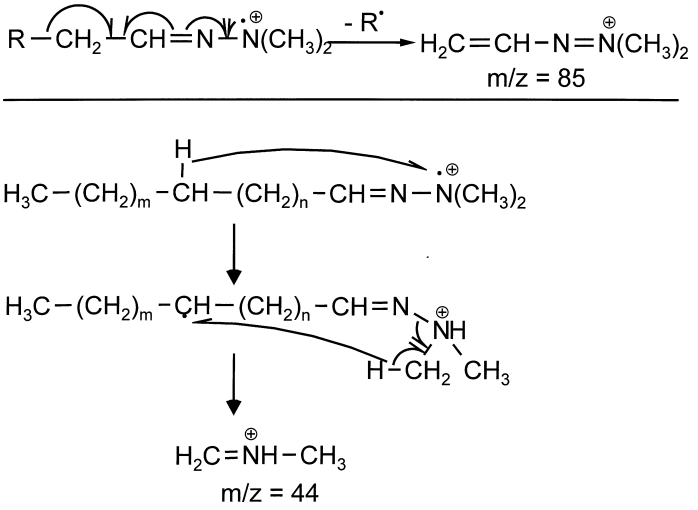


Fig. 22. Generation of the ions of m/z 85 and m/z 44 from dimethylhydrazones according to Djerassi [111] and Goldsmith [114].

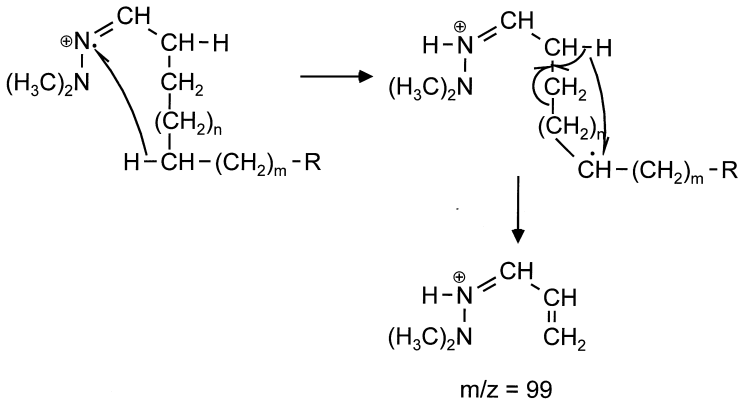


Fig. 23. Generation of ''McLafferty +13'' ions in analogy to Kraft and Spiteller [107].

In addition, their mass spectra do not allow more structural conclusions than those of other derivatives [115].

5.2. 2-Alkenals

The spectral characteristic of 2-alkenals are different compared to the corresponding saturated aldehydes (Fig. 5) in several respects: The ion of mass 44 (due to a McLafferty rearrangement induced by the -CH=O bond) is missing [116] while typical fragments of m/z 70 and 83 (Fig. 28) are generated probably in the course of a McLafferty rearrangement induced by the -HC=CH bond involving hydrogen migration to the double bond resp. by a double hydrogen rearrangement (Fig. 27):

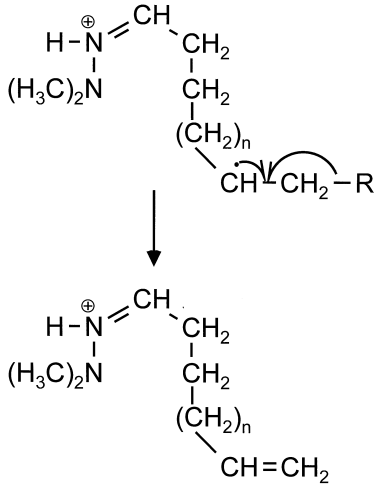


Fig. 24. Generation of fragments differing by 14 u of dimethylhydrazones derived from aldehydes.

The above outlined rearrangement reactions (Fig. 27) are also dominating degradation processes of corresponding methoximes (Fig. 29), leading to an ion of mass 112. In addition, cleavage in allylic position to the double bond is another important degradation path. The methoxime spectra of α,β-unsaturated aldehydes are characterized also by rather intense molecular ions, they show fragments 14 u apart (compare for Fig. 24), allowing to distinguish between the presence of a straight or branched (if corresponding ions are missing) carbon chain. Therefore mass spectra of methoximes derived from 2-alkenals provide more structural information than those of saturated aldehydes.

Equally the structural information of mass spectra obtained from pentafluorobenzyloxime derivatives is enhanced by introduction of an α,β double bond, thus spectra show molecular ions and, in addition to the key ion of mass 181, an ion which corresponds to the loss of the pentafluorobenzyl residue (M-181, m/z 140 in Fig. 31). Further, the α,β-double bond is recognized by another key ion of mass 250 [97], corresponding to formal cleavage of the bond between the β and γ carbon (see Fig. 31). In fact the generation of this ion starts after ionization of the double bond system by hydride migration followed

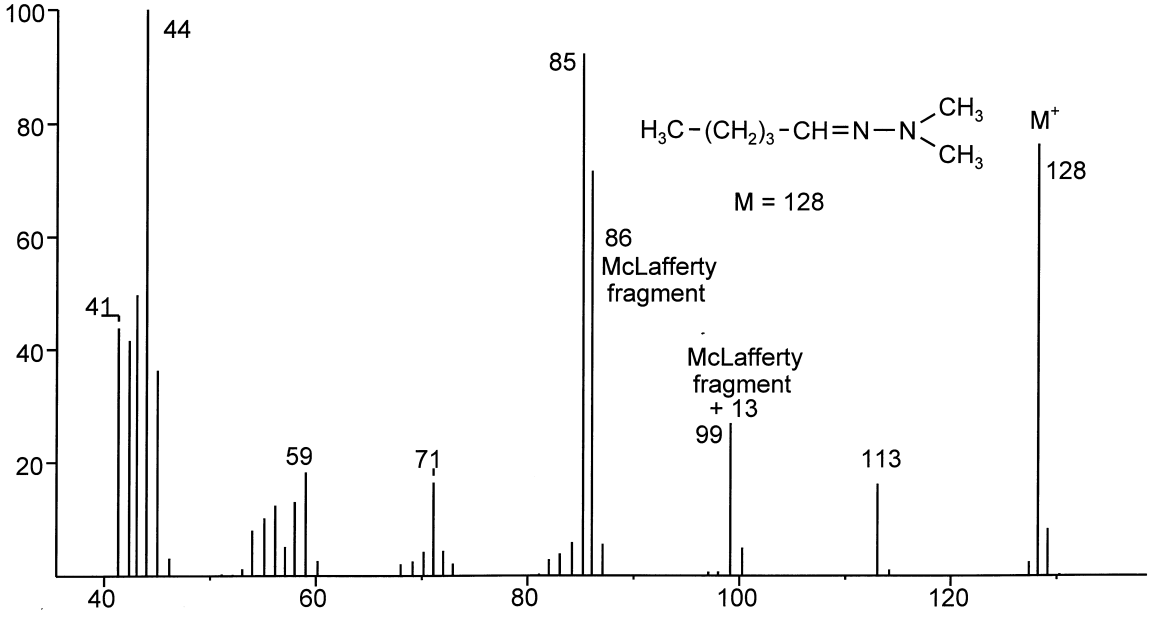


Fig. 25. Mass spectrum of the dimethylhydrazone of pentanal ($I=935$), see also Goldsmith and Djerassi [114].

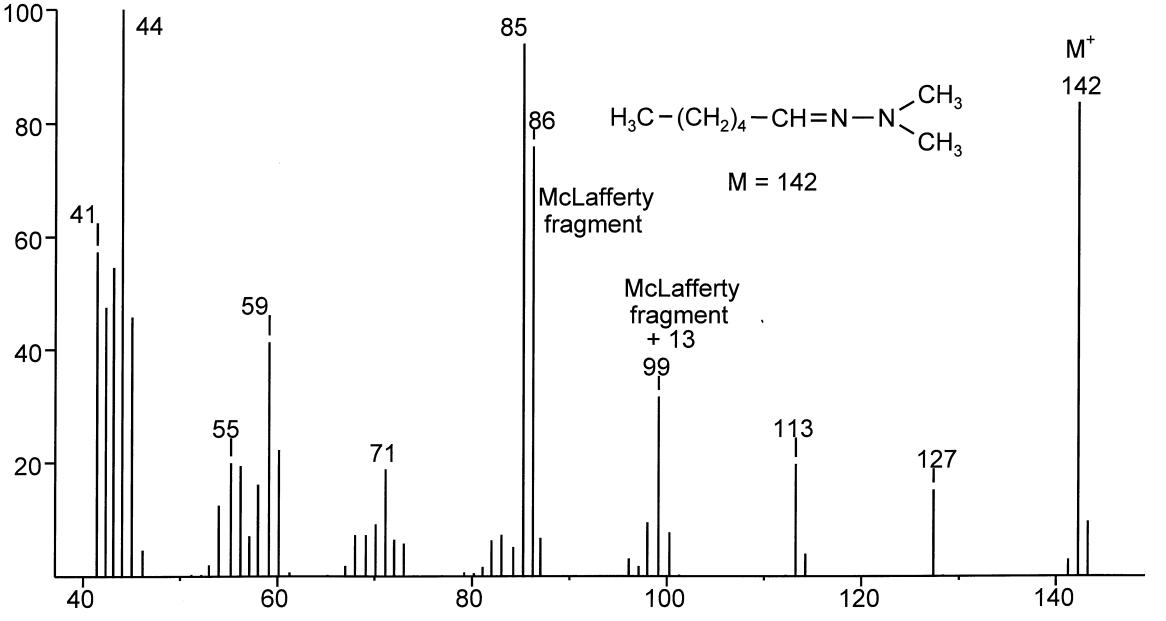


Fig. 26. Mass spectrum of the dimethylhydrazone of hexanal ($I=1035$), see also Goldsmith and Djerassi [114].

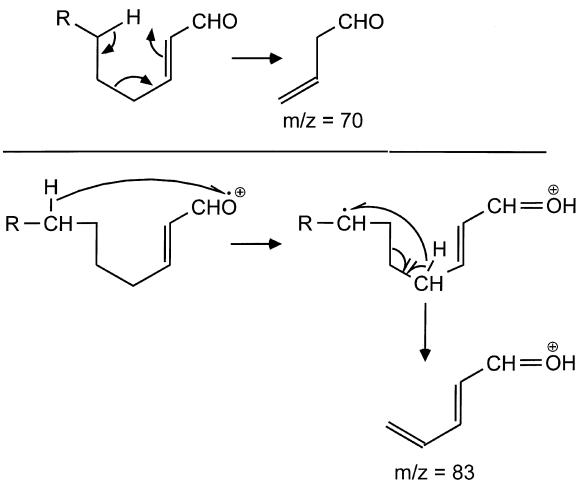


Fig. 27. Generation of main fragments in α,β-unsaturated aldehydes.

by cleavage of the C–C bond between C-2 and C-3 [117] (Fig. 30).

Thus pentafluorobenzyloxime derivatives are well suited for identification of α,β-unsaturated aldehydes.

Also the MSTFA adduct spectra of α,β-unsaturated aldehydes are very informative, since they do not indicate a few key ions only, but a series of fragments in an interval of 14 u (254, 268, 282, 296) which allow recognition that an alkyl chain is unbranched (Fig. 32, compare Fig. 24). Besides the typical fragments of MSTFA adducts are present, m/z 73, 75, 110, 134, 184, 199. The peak at m/z 228, the outstanding fragment in mass spectra of MSTFA adducts of saturated aldehydes, is often only of low abundance – since the tendency for cleavage at the adjacent bond to the original aldehyde group is reduced by the double bond, generation of alkenyl radicals requires much more energy than production of alkyl radicals [6,118].

The mass spectra of 2,4-dinitrophenylhydrazones of α,β-unsaturated aldehydes, e.g. of 2-octenal (Fig. 33) are represented by intense molecular ions. These are degraded by loss of an ·OH radical followed by loss of water (peaks m/z 289, resp. m/z 271 in Fig. 33). A main degradation path started with allylic cleavage followed by loss of water (m/z 231) and oxygen (m/z 215), alternatively the ion generated by allylic cleavage may be degraded further by expulsion of NO_2 (m/z 203). Another main fragment is produced by loss of the alkyl residue in the form of a saturated hydrocarbon (m/z 234). Thus the degradation is characterized by unexpected multistep

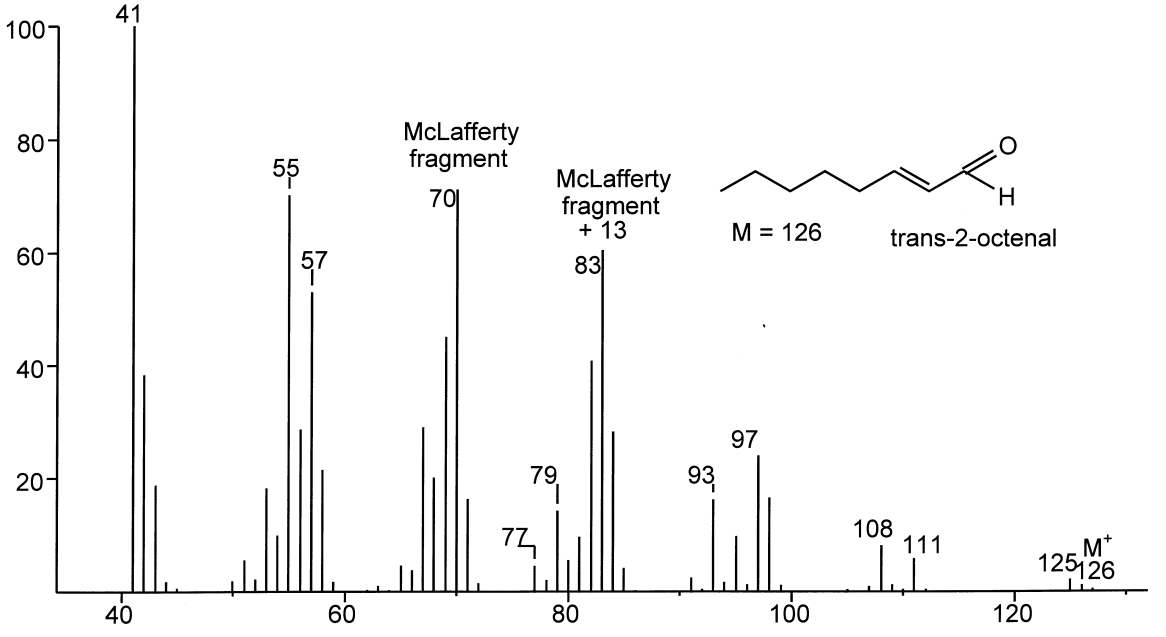


Fig. 28. Mass spectrum of *trans*-2-octenal ($I = 1018$).

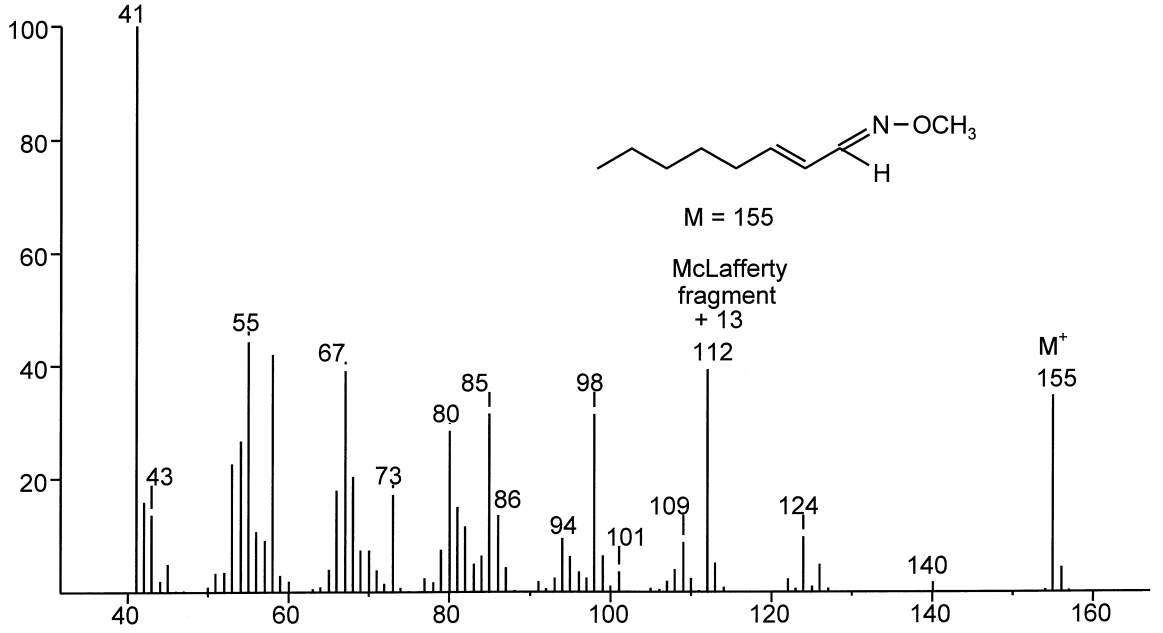


Fig. 29. Mass spectrum of the methoxime derived from *trans*-2-octenal ($I_1 = 1128$; $I_2 = 1146$).

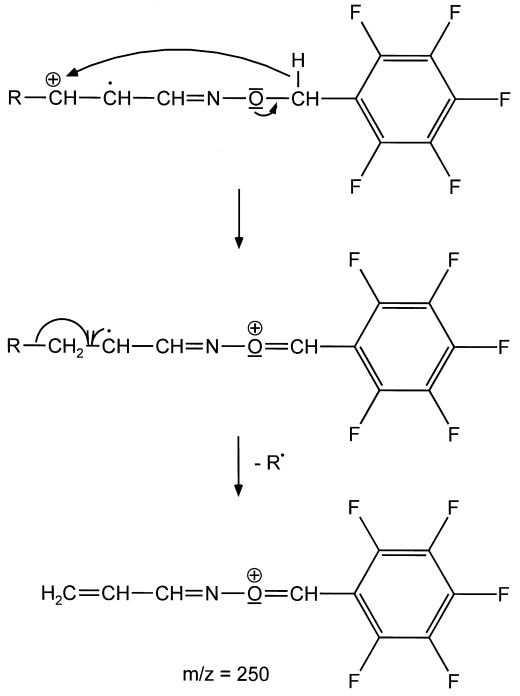


Fig. 30. Generation of the fragment m/z 250, derived from PFBO derivatives of 2-alkenals according to A. Loidl-Stahlhofen [117].

reactions, rendering the interpretation of the spectrum rather difficult (Fig. 33).

The MS of dimethylhydrazones of 2-alkenals show dominant molecular ions. Cleavage of the allylic bond generates a key ion of mass 111. Similarly as in mass spectra of dimethylhydrazones derived from saturated aldehydes a series of peaks every 14 u is recognized (Fig. 34). They are produced in an analogous way to the reactions outlined in Fig. 24).

As mentioned above, 2-methylpyrazolines are generated from α,β-unsaturated aldehydes [89] by addition of methylhydrazine (see Fig. 4). Their mass spectra produce also abundant molecular ions and α-fragments. Since all other derivatization reagents also provide informative mass spectra, the value of this special derivatization method is limited.

5.3. 2,4-Alkadienals

The introduction of a second double bond in conjugation to the aldehydic carbonyl group enhances the ionization probability more than in 2-alkenals, resulting in an increase of molecular ion peaks. The double bond system induces a prominent

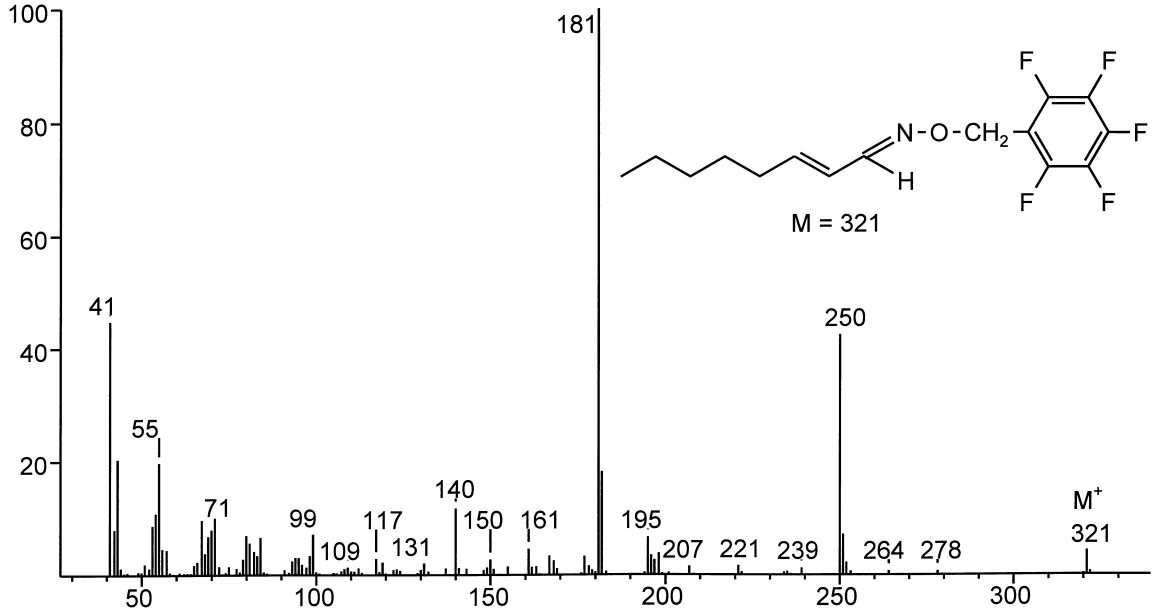


Fig. 31. Mass spectrum of the PFBO derivative of *trans*-2-octenal ($I_1 = 1693$; $I_2 = 1704$).

fragmentation reaction, leading to formation of a pyrylium ion (Fig. 35).

Thus the main fragment in the spectrum of 2,4-decadienal and 2,4-nonadienal is found at m/z 81 (Figs. 36 and 37) [119]. In addition, preferential cleavage in the allylic position is observed.

Derivatization of compounds with a 2,4-unsaturated carbonyl system is remarkably slower than that

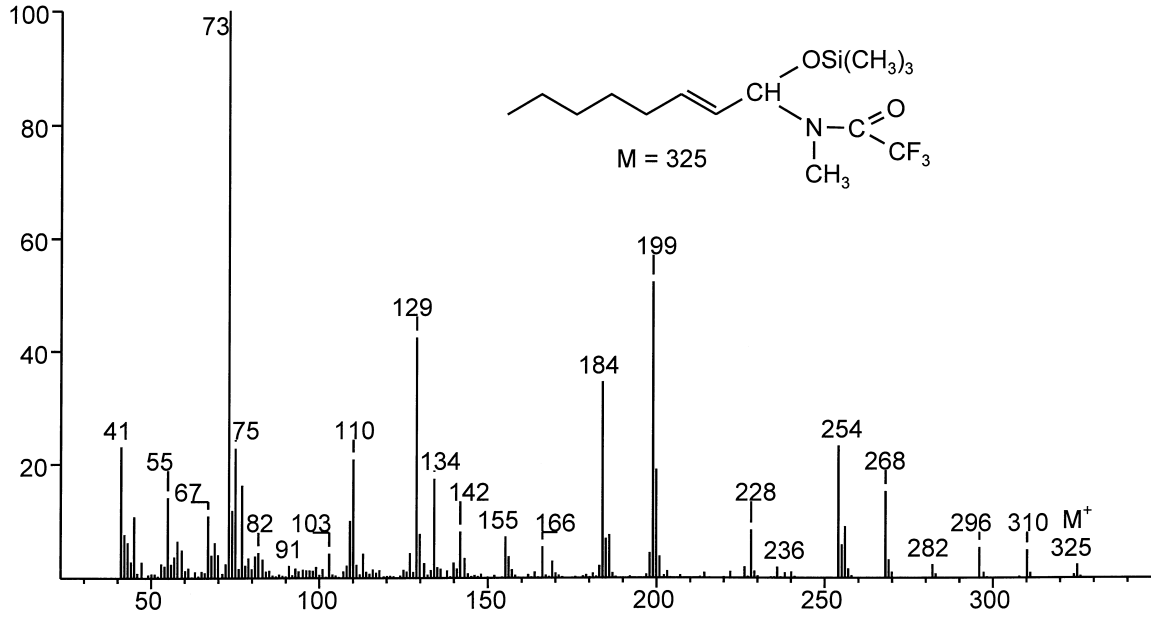


Fig. 32. Mass spectrum of the MSTFA adduct of *trans-2*-octenal ($I = 1477$).

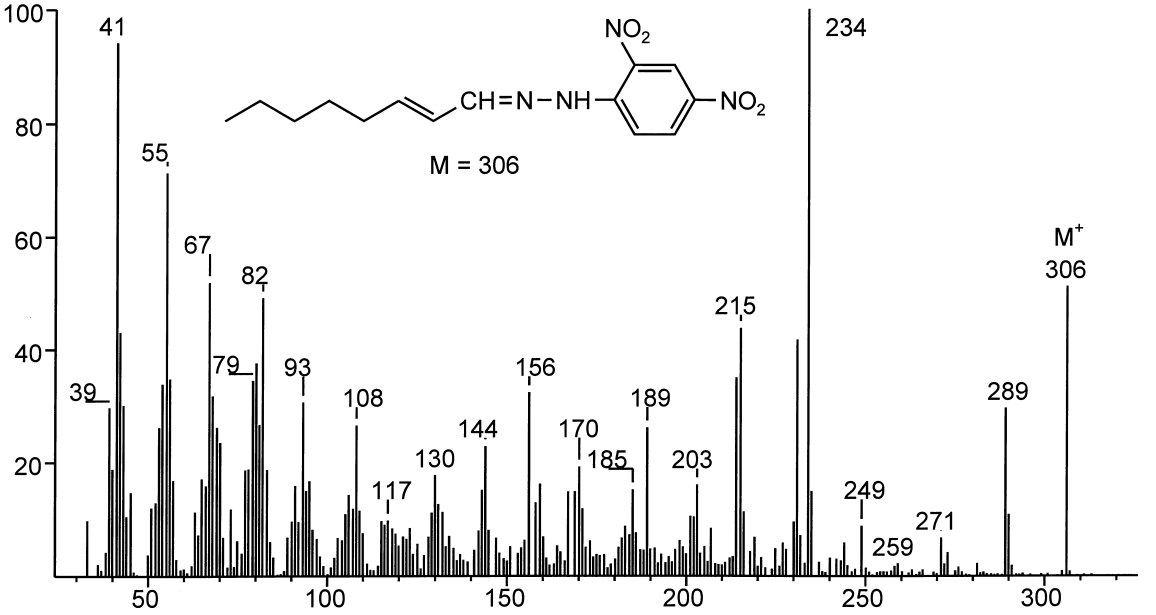


Fig. 33. Mass spectrum of the 2,4-dinitrophenylhydrazone of *trans*-2-octenal ($I = 2730$).

of saturated aldehydes due to the lower partial positive charge at the carbonyl carbon caused by resonance. As a consequence after derivatization often unchanged dienals are detected side by side with those which had reacted with the reagent. In addition, double bond isomerization causes generation of *E,Z* and *E,E*-diene isomers – besides *syn* and *anti* isomers. All isomers show nearly identical

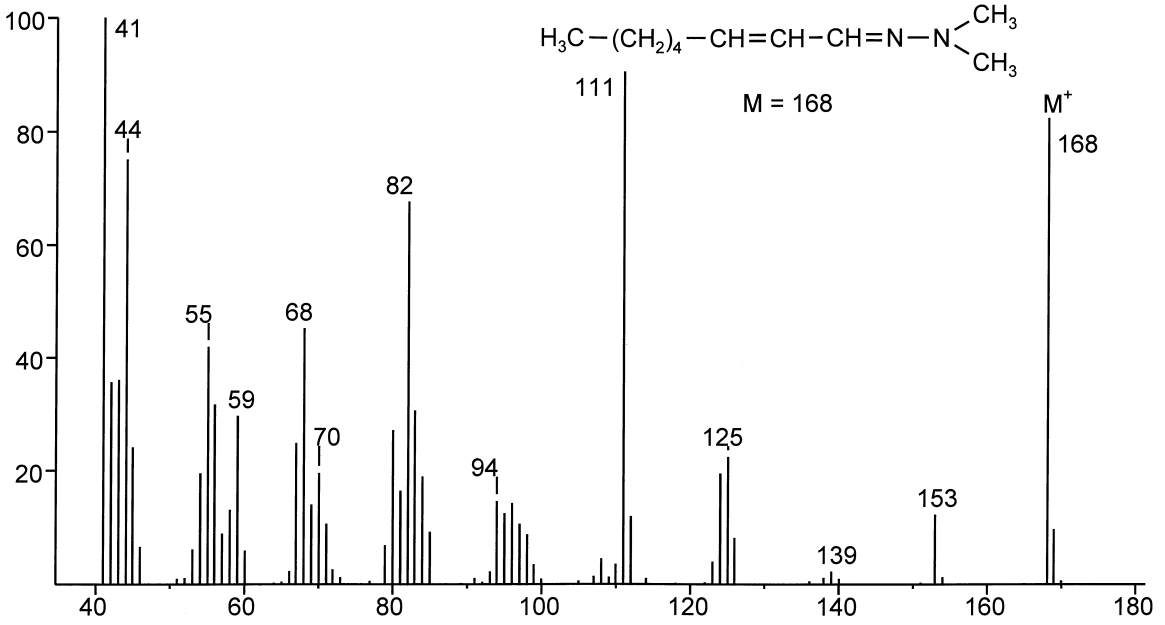


Fig. 34. Mass spectrum of the dimethylhydrazone of *trans*-2-octenal ($I = 1334$).

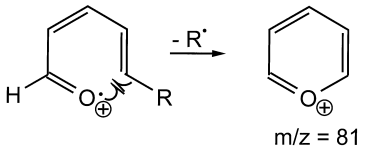


Fig. 35. Generation of pyrylium ions from 2,4-alkadienals.

spectra, they are distinguished by different *I* values.

The fragmentation behaviour of methoximes derived from 2,4-alkadienals resembles that of the underivatized aldehydes: The main fragment is a N-OCH_3 substituted pyridinium ion, generated in similar reaction as pyrylium ions from 2,4-alkadienals (Fig. 38):

The methoxypyridinium ion (m/z 110) is degraded by expulsion of formaldehyde to the pyridinium ion of mass 80 (Fig. 39).

Exactly the same main fragmentation routes are recognized for dimethylhydrazones and pentafluorobenzyloximes of 2,4-alkadienals: The main fragment of dimethylhydrazine derivatives of 2,4-alkadienals is generated by pyridinium ion formation (Fig. 40), followed by loss of $H_2C=N\text{-}CH_3$ instead of CH_2O (Fig. 38), while pentafluorobenzyloximes are degraded to an ion of mass 276 (Fig. 42).

The ion of m/z 181, the dominating ion in pentafluorobenzyl derivatives of saturated aldehydes, is obtained only in moderate abundance. Ions derived by allylic cleavage are observed (m/z 290 in Fig. 42), but only of low intensity.

The most prominent ion in the mass spectra of MSTFA adducts of 2,4-alkenals is found at m/z 308, corresponding to allylic cleavage: Ionization at the carbonyl oxygen induces a hydrogen shift from position 6. The generated ion triggers the cleavage of the C-7/C-8 carbon bond (Fig. 41).

Hydrogen migration to the carbonyl group does occur preferentially, but not exclusively, by hydrogen abstraction from C-6 (see Fig. 41). Thus, as outlined in Fig. 24, fragments are formed from a 2,4-dienal which are 14 u apart (Fig. 43). Besides CF_3 is lost, and the typical fragments for MSTFA derivatives (m/z 73, 110, 134, 184, M-126) are detected. Mass spectra of MSTFA derivatives prove therefore most useful for structural assignments.

5.4. 4,5-Epoxy-2-alkenals

4,5-Epoxy-2-alkenals are generated as outlined in Fig. 1 and also by epoxidation of 2,4-dienals by reaction with peroxylradicals [120].

Detection of epoxides is difficult, especially in

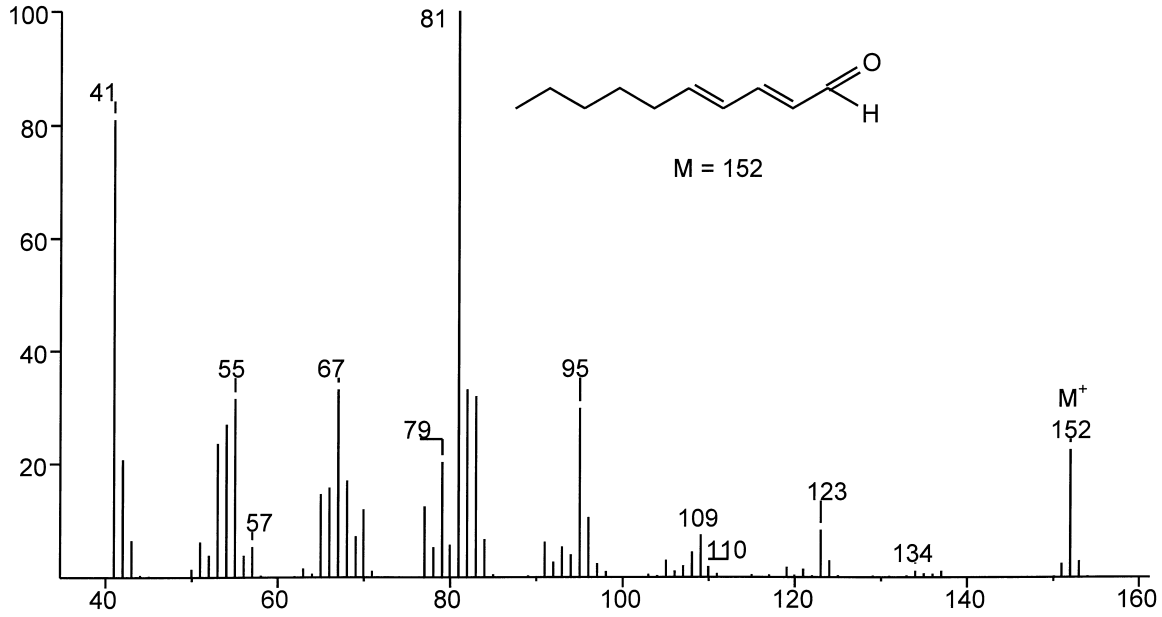


Fig. 36. Mass spectrum of *trans,trans*-2,4-decadienal (*I* = 1279).

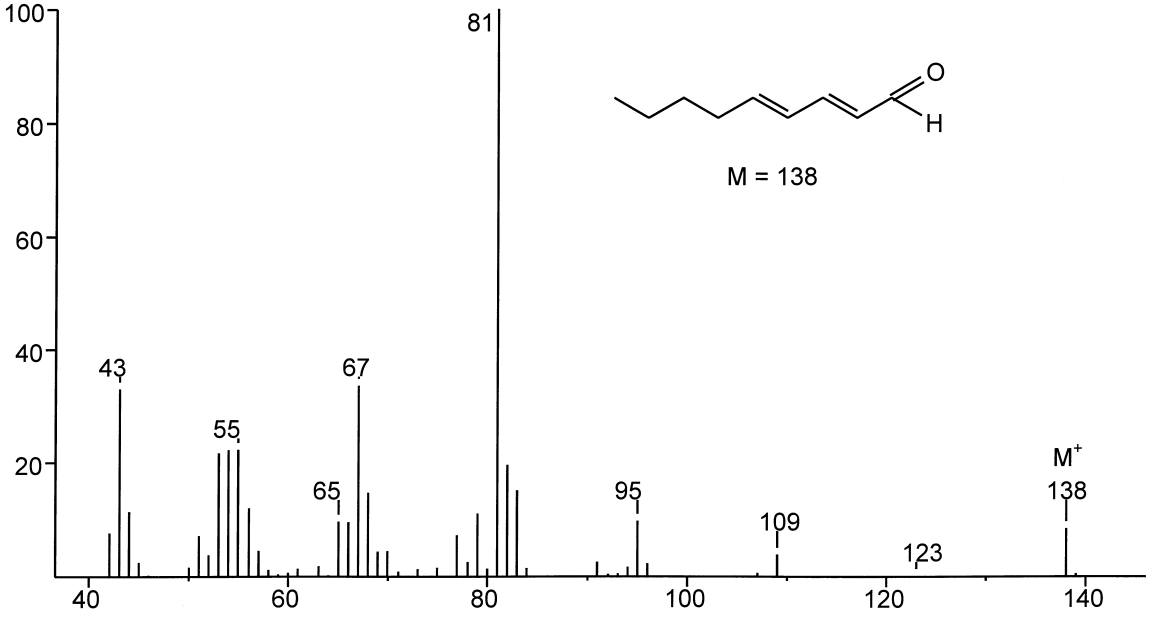


Fig. 37. Mass spectrum of *trans,trans*-2,4–nonadienal ($I = 1175$).

samples of biological origin, since their mass spectra usually show molecular ions of very low intensity only, if at all. Spectra of 4,5-epoxy-2-alkenals are characterized by ions of high intensity which are derived by formal cleavage across the epoxide ring (Fig. 44) causing the loss of an aldehyde molecule. This reaction generates a furan ion of m/z 68 (Fig. 45).

Attempts to derivatize epoxy aldehydes with carbonyl trapping reagents revealed that a great number of products is obtained, obviously the aldehyde group reacts not only in the expected manner, but also reagent is added by opening of the epoxide ring. If acid is used to enhance the reactivity of the carbonyl group water may be added also at the epoxide function. In addition, mass spectra of the derivatives are not very informative, as demonstrated by the spectrum of the PFBO derivative of 4,5-epoxy-2-decenal (Fig. 46). Nevertheless an ion at M-197 allows to deduce the molecular mass, obviously oxygen is lost from the molecular ion (peak at M-16) forming the PFBO derivative of a 2,4-alkadienal. This reaction is recognized by the key ion of mass 276, characteristic for these types of compounds (compare Fig. 27).

Nearly all 4,5-epoxy-2-enal derivatives show in their spectra loss of a fragment corresponding to the epoxy part of the molecule in form of an aldehyde, as demonstrated with the spectra of the double bond isomeric 4,5-epoxy-2-decenals (Fig. 46): These are characterized by a peak corresponding to expulsion of $C_5H_{11}CHO$ (M-100), see Fig. 44.

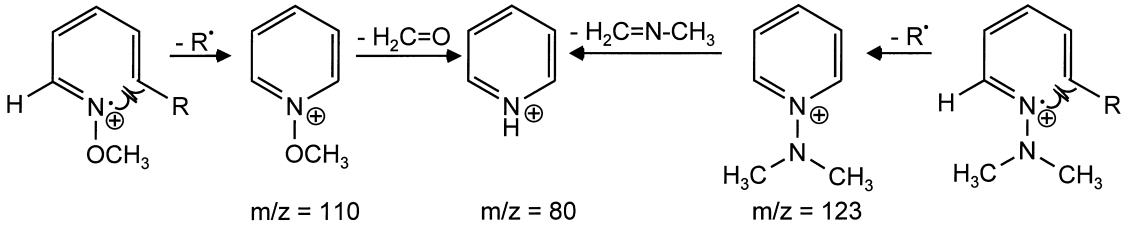


Fig. 38. Generation of pyridinium ions from methoximes and dimethylhydrazones derived from 2,4-alkadienals.

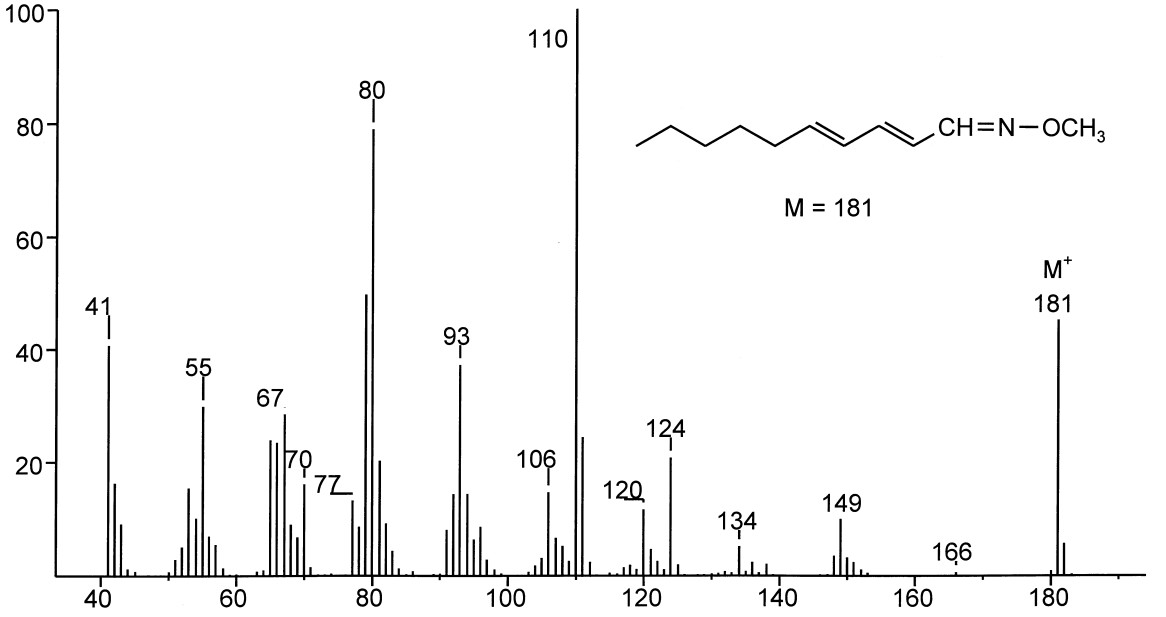


Fig. 39. Mass spectrum of the methoxime of *trans,trans*-2,4-decadienal ($I_1 = 1396$; $I_2 = 1420$).

MSTFA adducts of 4,5-epoxy-2-enals are generated more readily than those of oximes and hydrazones, but their mass spectra are also not very informative, molecular ions are missing, characteristic fragment ions are generated by the cleavage process outlined in Fig. 44, by formal cleavage across the epoxide ring. In addition, an ion indicates loss of the alkyl residue (M−71 in Fig. 47).

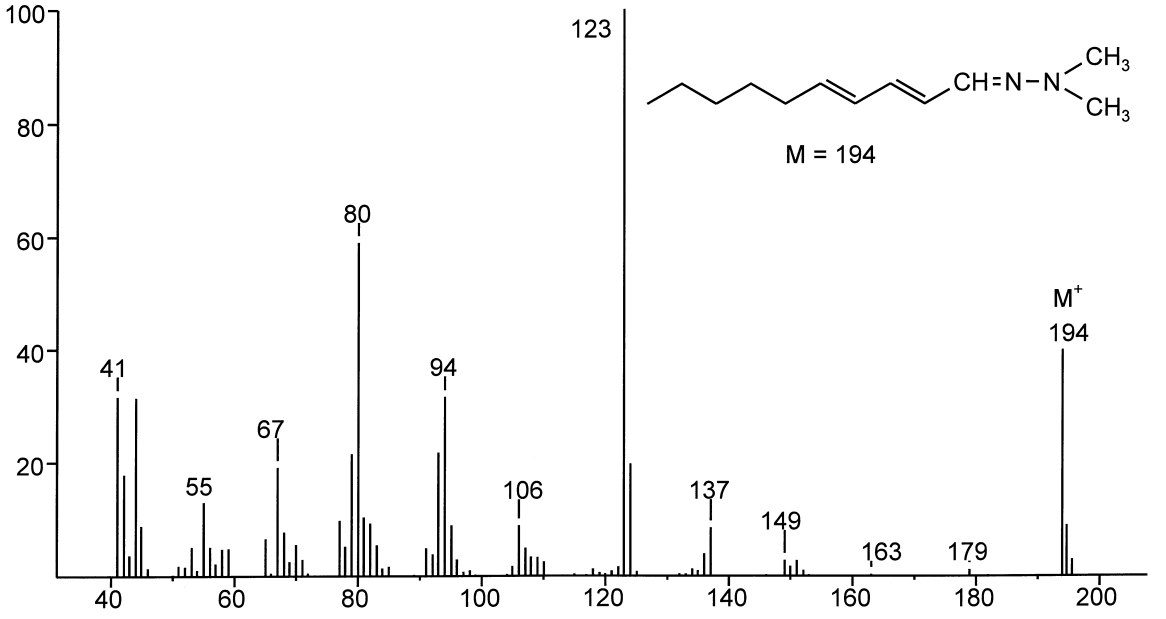


Fig. 40. Mass spectrum of the dimethylhydrazone of *trans,trans*-2,4-decadienal ($I = 1614$).

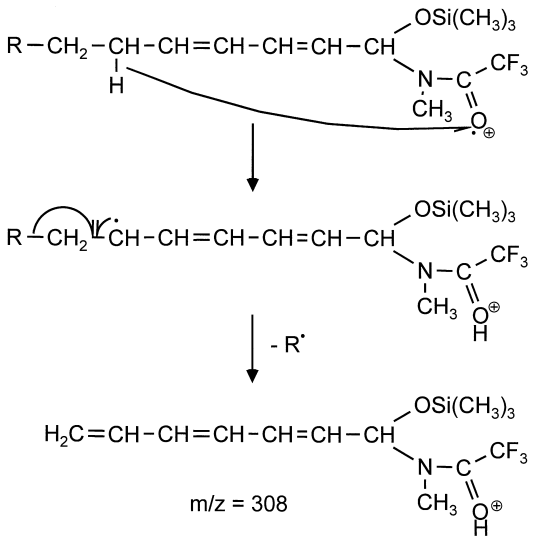


Fig. 41. Main fragmentation route of MSTFA adducts derived from 2,4-dienals.

5.5. 4-Hydroxy-2-alkenals

As outlined in Fig. 3, hydroperoxides undergo further oxidation. In the course of these reactions, 2-hydroxyaldehydes and 4-hydroxy-2-alkenals are generated [87,108,124] 4-Hydroxy-2-alkenals suffer easily dehydration, consequently protection of the hydroxy function for a GC analysis is required. By short treatment with MSTFA the OH group is transformed to a OTMS group, prolonged treatment converts 4-trimethylsilyloxy-2-alkenals to corresponding MSTFA adducts. Thus both derivatives may be found together.

The mass spectrum of 4-trimethylsilyloxy-2-nonenal (Fig. 49) is characterized by an intense α-cleavage fragment m/z 157 [26], which decomposes further by loss of CO to a fragment m/z 129. The molecular ion is detectable, but only of low abundance. Additional fragments arise by loss of CH_3 or CHO from the molecular ion (Fig. 48).

In the mass spectrum of the MSTFA adduct of 4-trimethylsilyloxy-2-nonenal (Fig. 50) an ion of mass 356 is dominating, produced by α-cleavage. The typical MSTFA ions of mass 228, 184, 134 and 110 are of low abundance only. Presence of two TMS residues in the molecule is indicated by the peak of mass 147, representing the fragment [125]:

$$(H_3C)_2Si=\overset{\oplus}{O}-Si(CH_3)_3$$

A molecular ion is missing, but deducible from an ion of mass 412 indicating the loss of a methyl

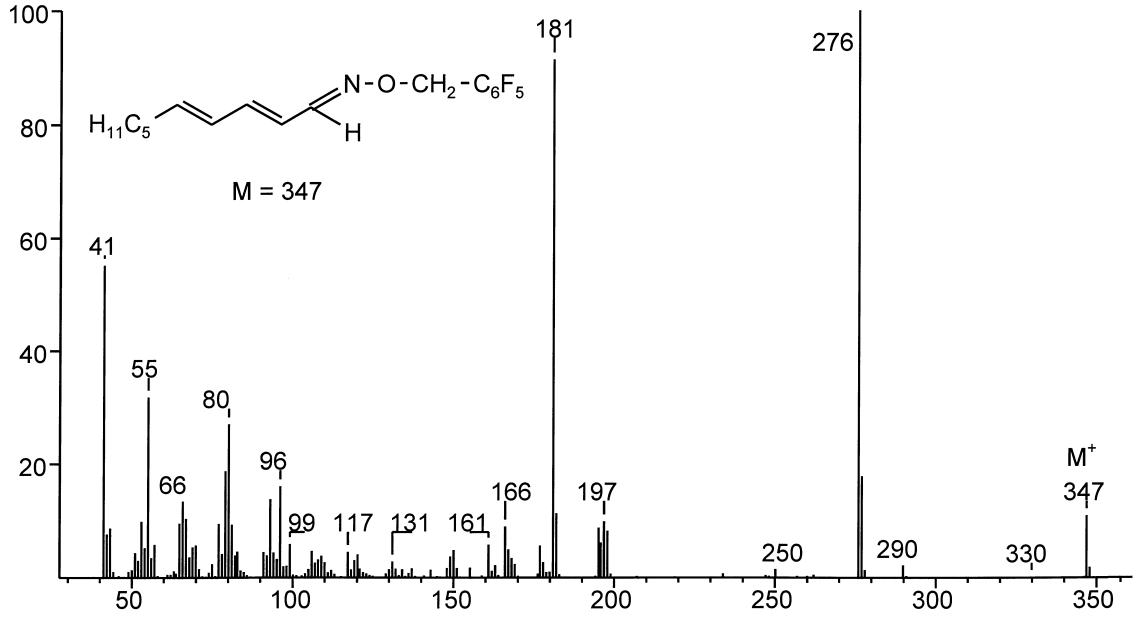


Fig. 42. Mass spectrum of PFBO of *trans,trans*-2,4-decadienal ($I_1 = 1971$; $I_2 = 1977$).

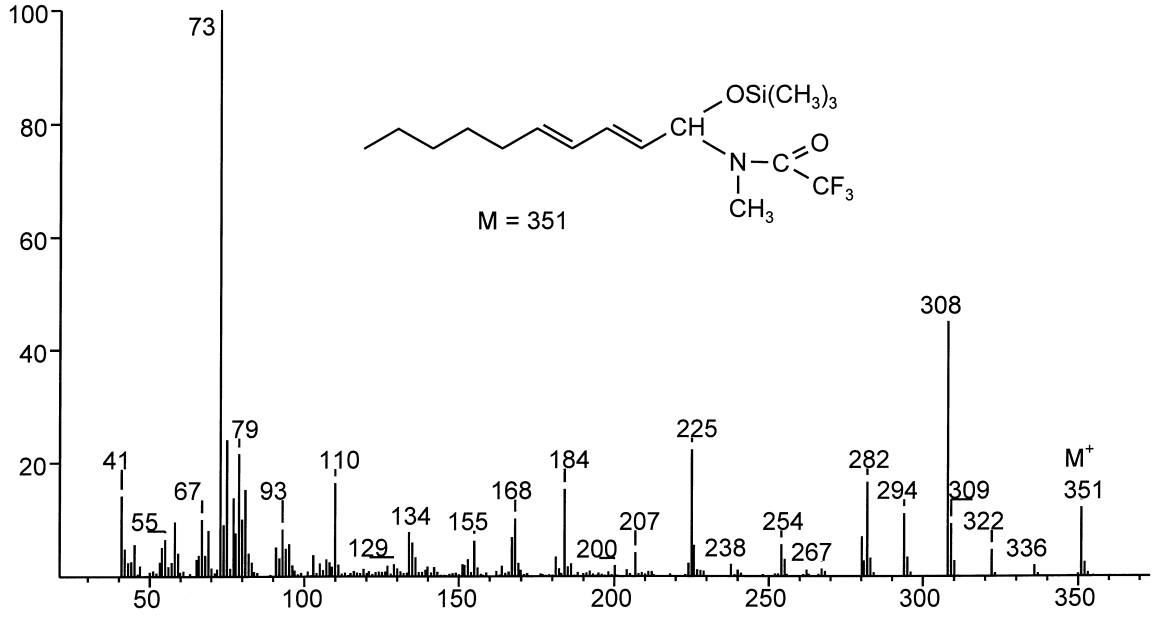


Fig. 43. Mass spectrum of the MSTFA adduct of *trans,trans*-2,4-decadienal ($I = 1718$).

group, and an ion, corresponding to loss of $CH_3N^{\cdot}$-$COCF_3$ (m/z 301). This ion is accompanied by an ion of mass 300, generated by expulsion of CH_3-N= C(OH)-CF_3. It suffers loss of a methyl group (m/z 285).

α-Fragmentation is also the main cleavage reaction in the mass spectrum of the PFBO derivative of 4-trimethylsilyloxy-2-nonenal (Fig. 51). Thus a prominent ion of mass 352 is generated, corresponding to the loss of the pentyl residue. In addition, M-15 (m/z 408), M-181 (m/z 242) and M-197 (m/z 226) ions are main fragments. The molecular ion is just detectable.

Since 4-hydroxy-2-alkenals possess a double bond in α-position, they react with methylhydrazine to pyrazolines (Fig. 4), their spectra may also be used for identification [89,90].

As pointed out already, trimethylsilylethers are partially hydrolyzed when subjected to chromatography by TLC. Therefore occasionally *tert*.-butyldimethylsilyl derivatives are prepared [80–82]. These show in their mass spectra dominant peaks corresponding to loss of $(H_3C)_3C^{\cdot}$ (M-57). These derivatives are especially useful to deduce the molecular mass.

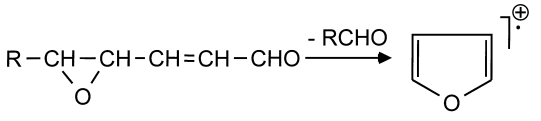


Fig. 44. Loss of aldehydes from 4,5-epoxy-2-alkenals.

5.6. 2-Hydroxyalkanals

Investigation of 2-hydroxyalkanals by GC–MS requires also protection of the hydroxy group in order to avoid thermal dehydration. Thus for instance 2-hydroxyheptanal is degraded to 2-heptenal, often reported as LPO product [27,83,118,126]. Protection is achieved by derivatization with MSTFA, in the first step OTMS derivatives are generated, which are further transformed to MSTFA adducts.

The mass spectrum of 2-trimethylsilyloxyheptanal is characterized by a strong key ion of mass 173. This suffers loss of C_5H_{10} – a typical secondary degradation reaction of trimethylsilyloxy ions – to an ion of mass 103. A fragment at M-15 allows the deduction of the molecular mass (Fig. 53).

The mass spectrum of the corresponding aldehyde adduct (Fig. 54≠) shows the α-cleavage product of mass 173 only with moderate abundance, obviously

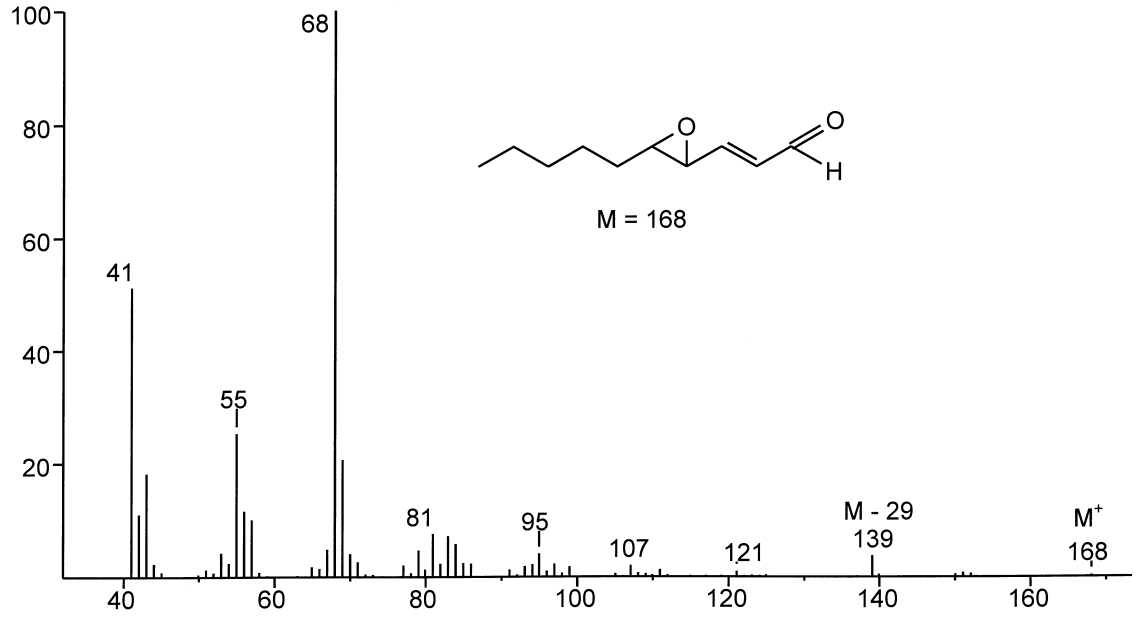


Fig. 45. Mass spectrum of 4,5-epoxy-2-decenal ($I = 1336$) [121–123].

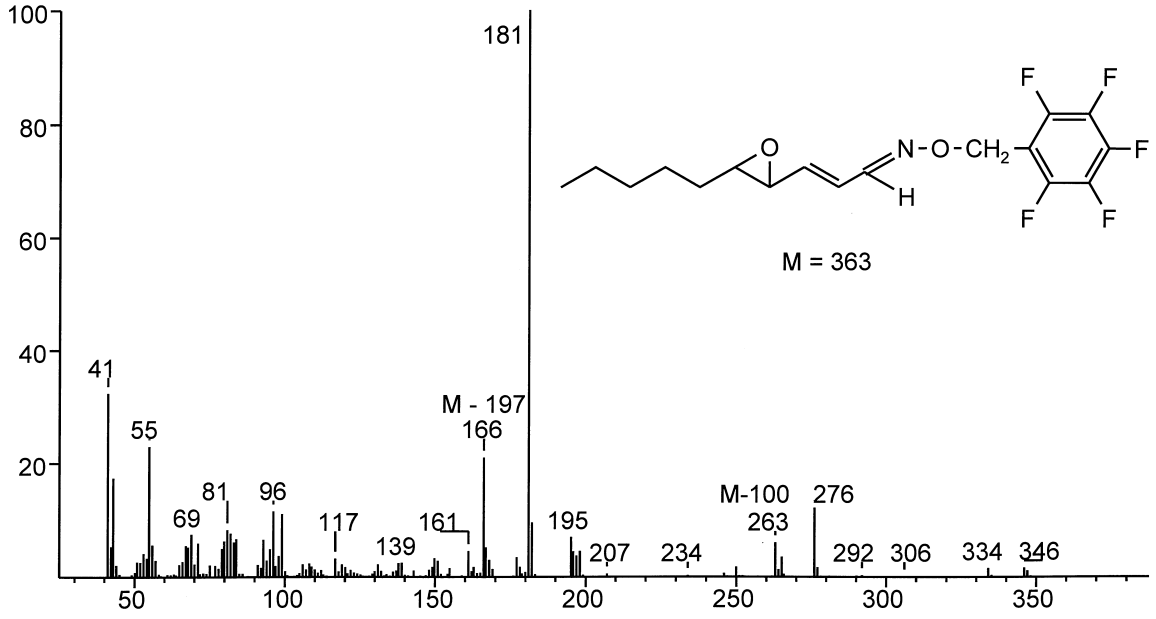


Fig. 46. Mass spectrum of the PFBO derivative of 4,5-epoxy-2-decenal ($I_1 = 2033$; $I_2 = 2054$).

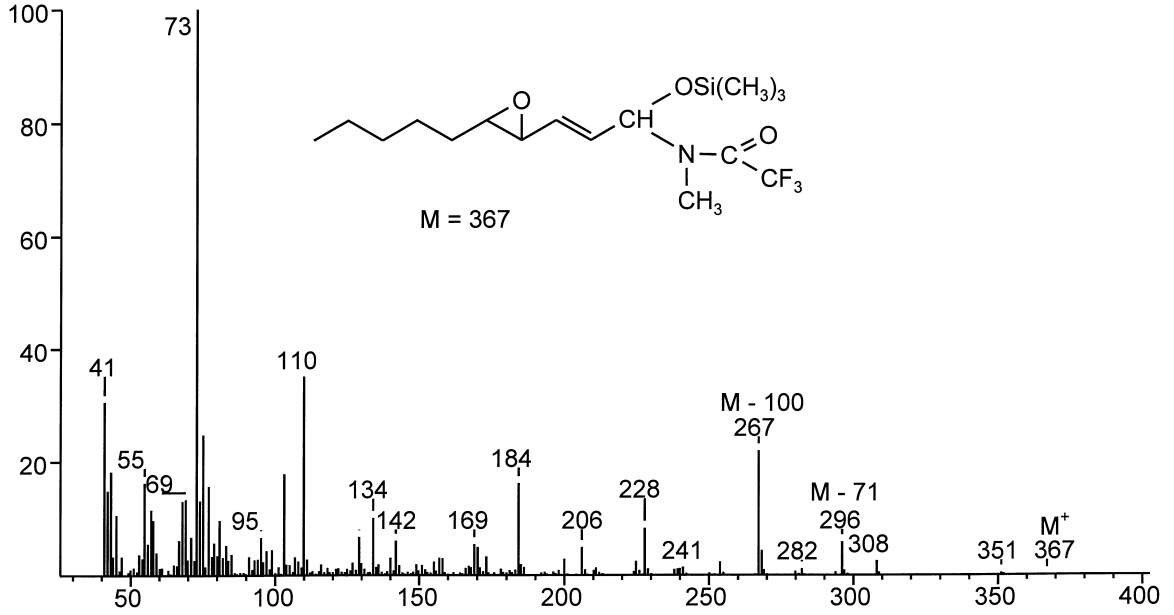


Fig. 47. Mass spectrum of the MSTFA adduct of 4,5-epoxy-2-decenal ($I = 1807$).

the charge remains mainly on the alternative α-cleavage product, the ion of mass 228. In addition, the spectrum is characterized by an ion M-100 (m/z 301). It is generated by loss of hexanal, requiring migration of the TMS residue to the carbonyl function as outlined in Fig. 52. Such processes are also common for trimethylsilyloxymethylesters and trimethylsilylesters derived from aldehydic acids and their derivatives [127,129].

The preparation of other derivatives of 2-hydroxy-aldehydes requires two derivatization steps: First the aldehydic group is reacted with a carbonyl reagent, subsequently the obtained derivative is treated with MSTFA to protect the OH group [87,97].

Trimethylsilylated PFBO derivatives of 2-hydroxyaldehydes suffer α cleavage, but in contrast to MSTFA adducts (main cleavage between C-1/C-2) the bond between C-2 and C-3 is broken preferentially to an ion of outstanding intensity (m/z 326) which is further degraded by loss of CO. Besides M-15 and M-197 ions are generated, allowing firm compound identification (Fig. 55).

5.7. 6-Hydroxy-2,4-dienals

As expected the main degradation reaction of 6-trimethylsilyloxy-2,4-undecadienal occurs by α-cleavage (Fig. 56). In contrast the dominant cleavage

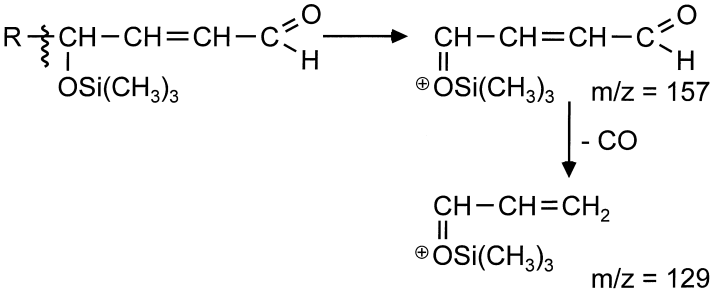


Fig. 48. Generation of the ion of mass 157 in trimethylsilyloxy-2-alkenals.

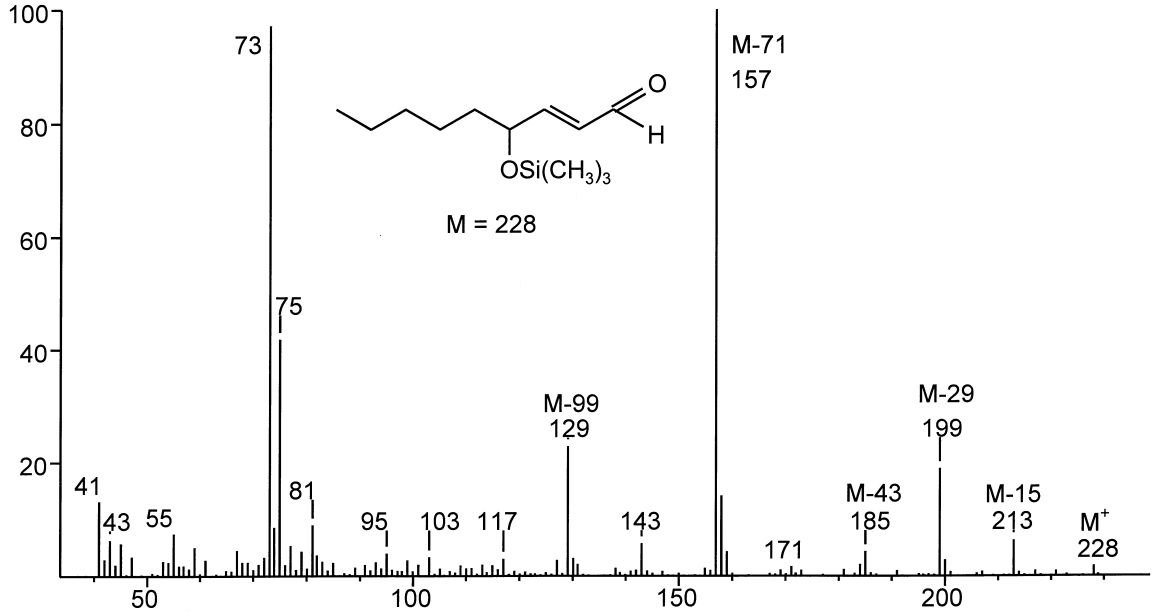


Fig. 49. Mass spectrum of the TMS derivative of 4-hydroxy-2-nonenal* ($I = 1392$*).

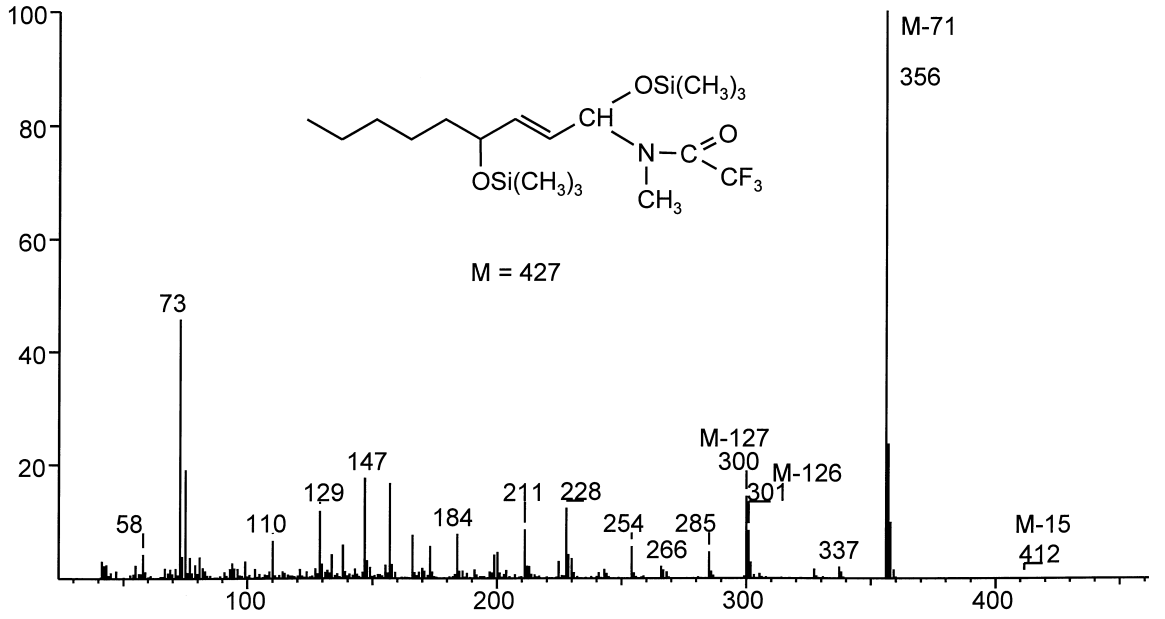


Fig. 50. Mass spectrum of the TMS derivative of the MSTFA adduct of 4-hydroxy-2-nonenal* ($I_1 = 1699$*; $I_2 = 1702$*).

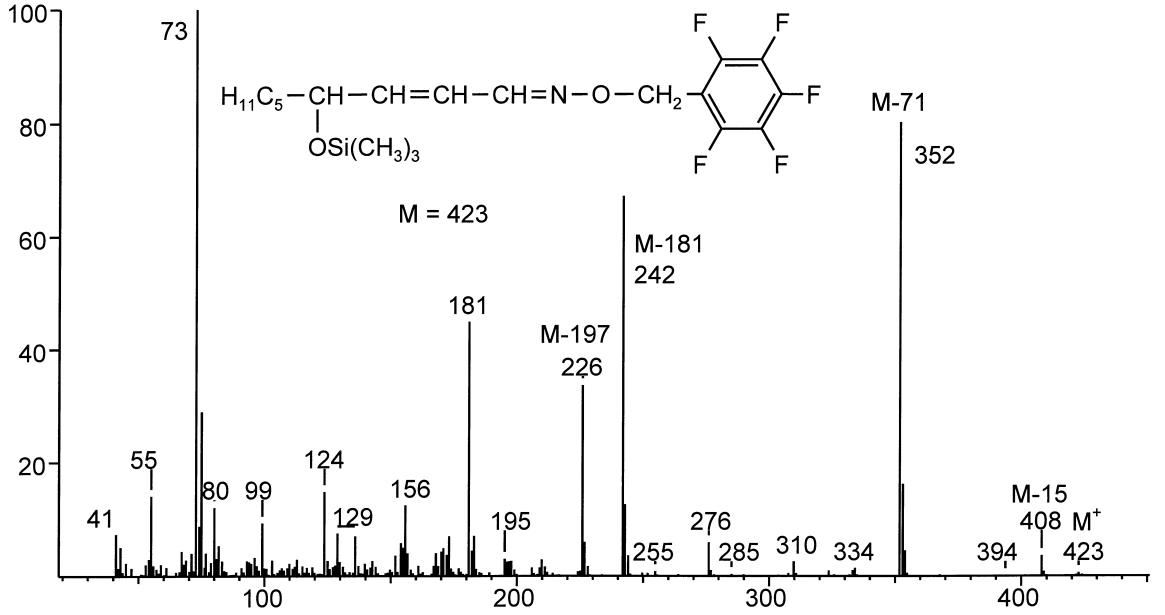


Fig. 51. Mass spectrum of the pentafluorobenzylhydroxylamine derivative of 4-trimethylsilyloxy-2-nonenal* ($I_1 = 1981$*; $I_2 = 1987$*), see also Mlakar [128].

reaction in the mass spectrum of its trimethylsilylated pentafluorobenzyloxime derivative (Fig. 57) does not occur by α-cleavage, but involves loss of a pentafluorobenzyl radical to form a pyrylium ion of mass 252 (compare Fig. 35) and the α-cleavage adjacent to the OTMS group is only of moderate importance (m/z 378) [97].

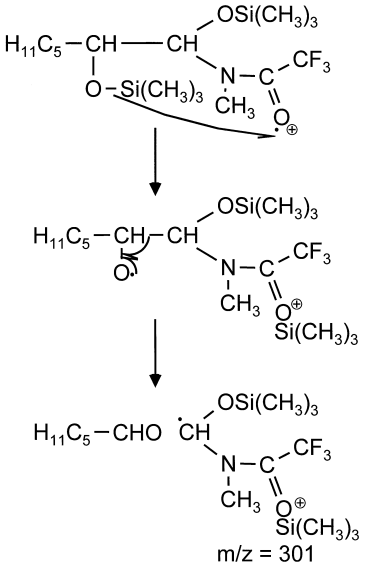


Fig. 52. Rearrangement of the TMS group in 2-trimethylsilyloxyheptanal and its degradation to hexanal, according to Mlakar [108].

5.8. Vicinal dihydroxyaldehydes

Vicinal dihydroxyaldehydes are generated by hydrolysis of epoxides, e.g. 4,5-dihydroxy-2-alkenals are obtained from 4,5-epoxy-2-enals. Since hydroxy compounds usually do not pass the gas chromatograph without thermal decomposition, protection of the hydroxy groups is required, e.g. by trimethylsilylation. If MSTFA is used for this purpose the aldehyde group may react in a second step by adduct formation. This reaction produces several stereoisomers, indicated by several peaks in the GC. The isomers show nearly identical mass spectra.

The mass spectrum of the adduct derived from trimethylsilylated 4,5-dihydroxy-2-decenal (Fig. 59) [35] shows as major peak at m/z 173 for an ion, arising by cleavage between the carbons carrying the OTMS residues. Interestingly the corresponding α-fragment is not detected, but an intense ion of mass 230, generated in a double shift of OTMS-residuals

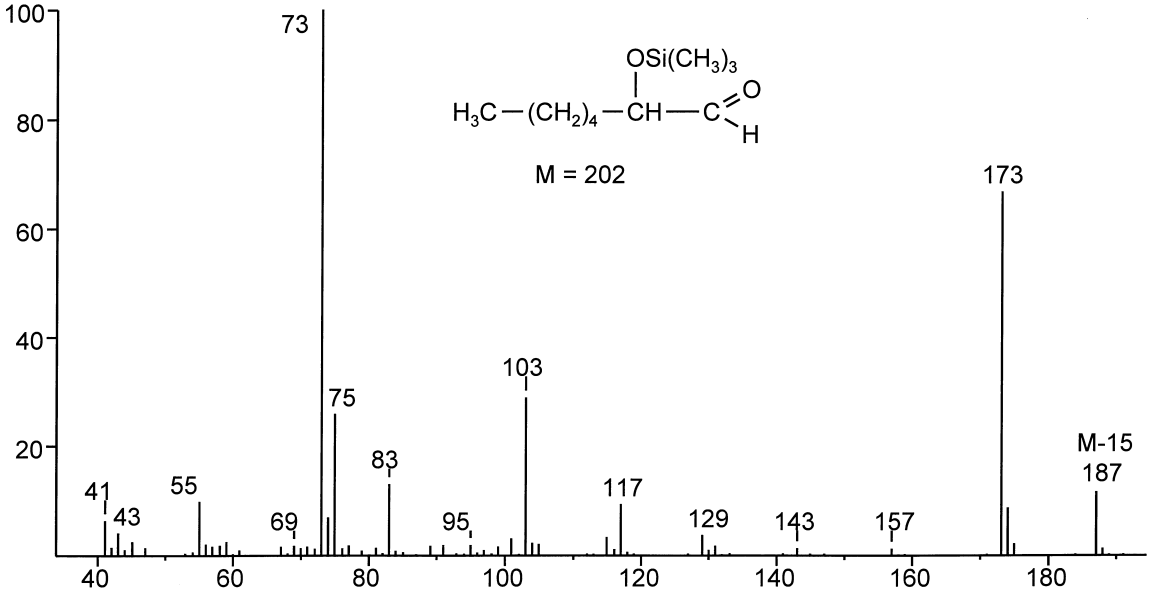


Fig. 53. Mass spectrum of the TMS derivative of 2-hydroxyheptanal* ($I = 1134$*).

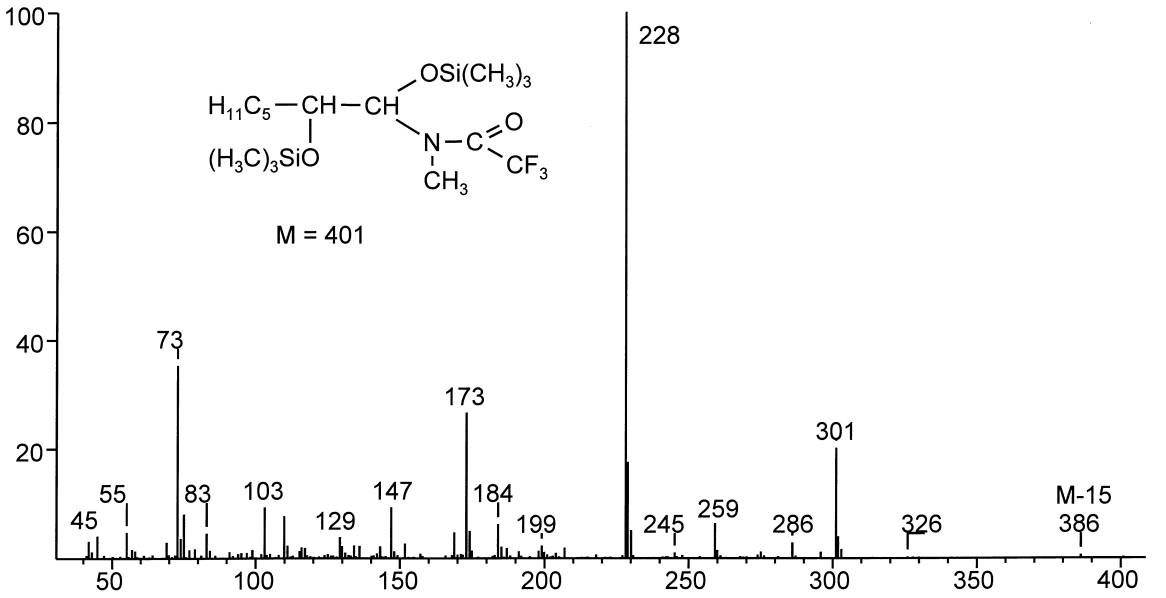


Fig. 54. Mass spectrum of the MSTFA adduct derived from 2-trimethylsilyloxyheptanal* ($I = 1538$*), see Loidl-Stahlhofen [87].

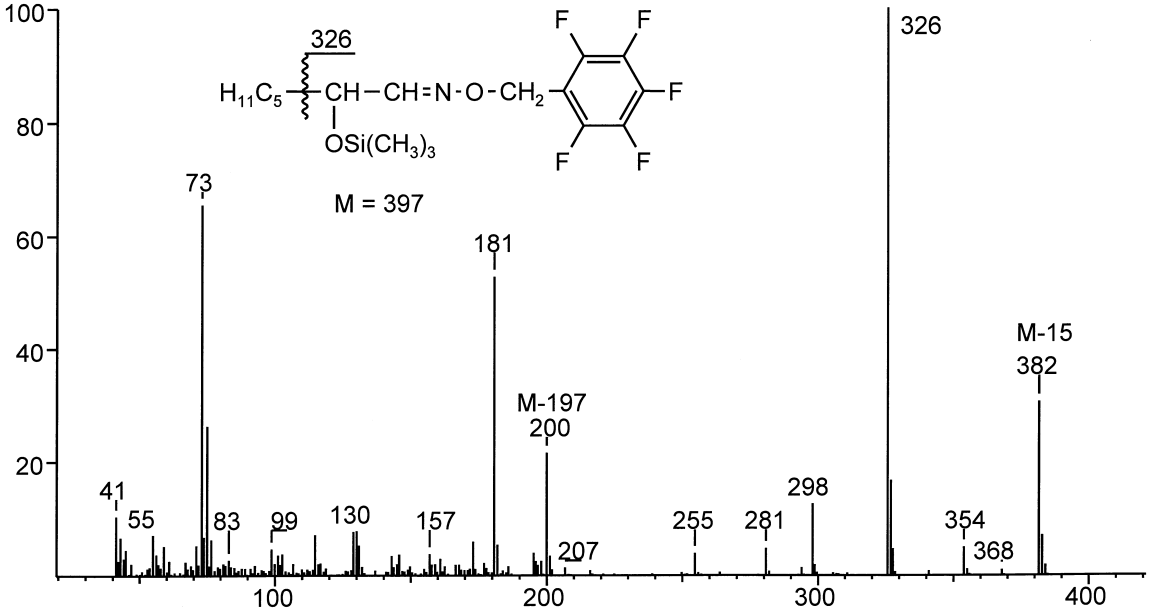


Fig. 55. Mass spectrum of the PFBO derivative of 2-trimethylsilyloxyheptanal* ($I = 1704$*), see Loidl-Stahlhofen [87].

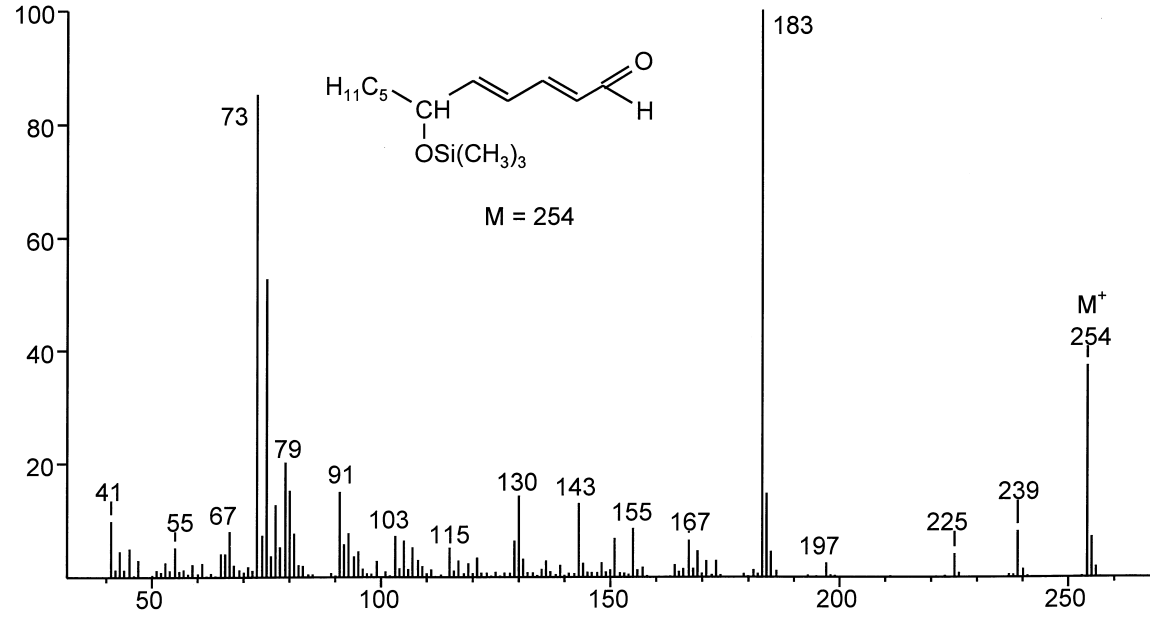


Fig. 56. Mass spectrum of the TMS derivative of 6-hydroxy-2,4-undecadienal* ($I = 1578,1628$*), see Loidl-Stahlhofen et al. [97].

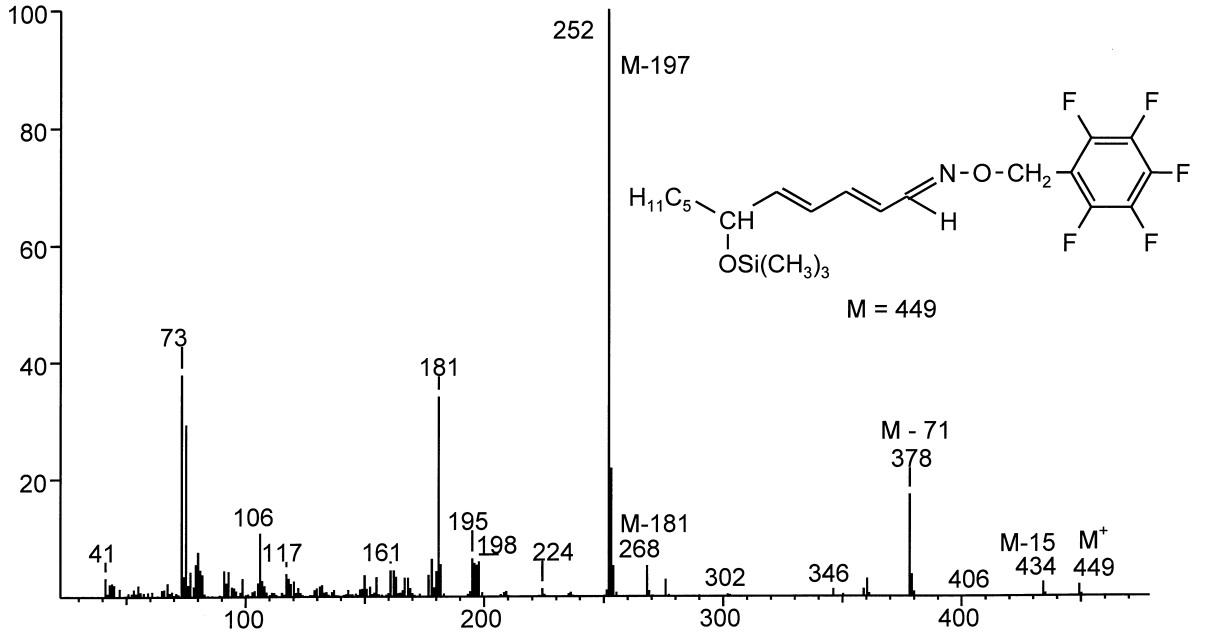


Fig. 57. Mass spectrum of the PFBO/TMS derivative of 6-hydroxy-2,4-undecadienal* ($I_1 = 2269$*; $I_2 = 2304$*), see Loidl-Stahlhofen [97].

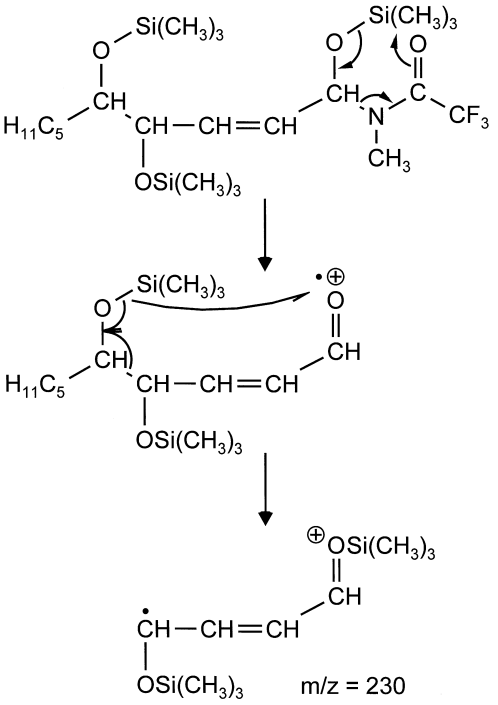


Fig. 58. Double McLafferty like reaction to form a fragment of mass 230 from the MSTFA adduct of 4,5-*bis*-trimethylsilyloxy-2-decenal.

as suggested by J. Reiner (University Bayreuth, personal communication) (Fig. 58).

5.9. Aldehydic acids

Compounds related to the foregoing are generated instead from 13-hydroperoxy-9,11-octadecadienoic acid from 9-hydroperoxy-10,12-octadecadienoic acid. As a consequence they contain an acidic component, $(CH_2)_7$-COOH, instead of the alkyl residue (C_5H_{11}).

These compounds are nonvolatile and require before GC–MS investigation derivatization of the carboxylic group. In principle the main degradation routes of derivatives of aldehydic acid esters follow the degradation paths of analogous derivatives of saturated aldehydes discussed above. The residue $(CH_2)_7$-$COOCH_3$ is 86 u heavier than the C_5H_{11} residue, as a consequence all ions containing the original acidic function are shifted in the spectra by 86 u. Mass spectra of methylates show in addition some structural specific ions, although often of low intensity only, indicating the presence of the $COOCH_3$ group, e.g. α-cleavage products (M-31 ions and ions of mass 59 ($COOCH_3^{\oplus}$).

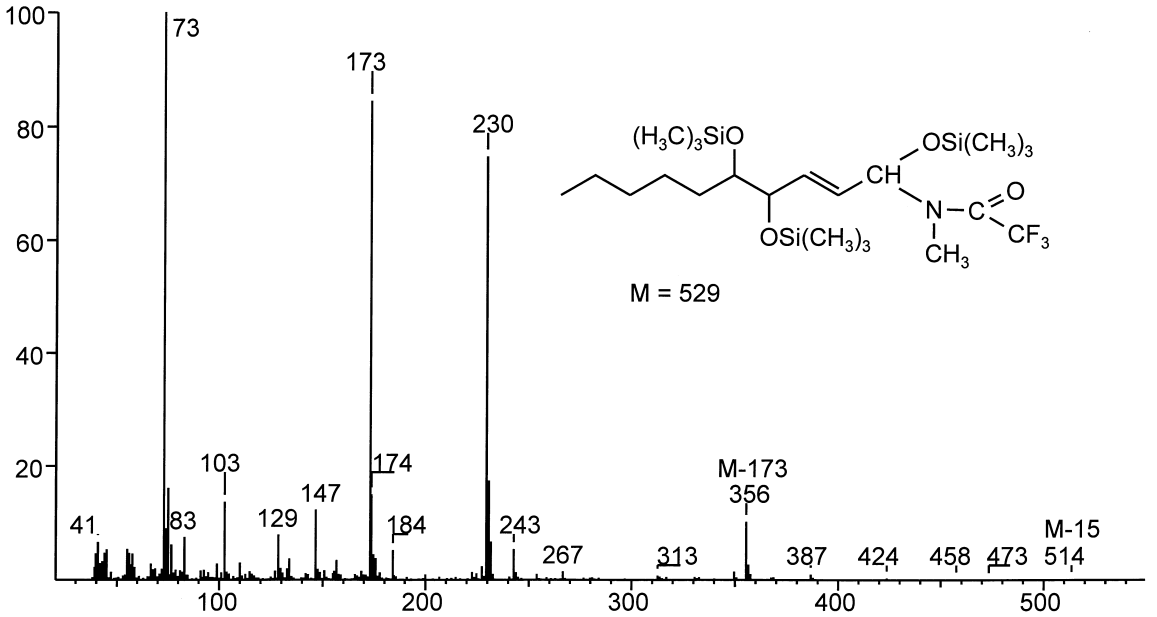


Fig. 59. Mass spectrum of the MSTFA adduct derived from 4,5-*bis*-trimethylsilyloxy-2-decenal ($I_1 = 1970$; $I_2 = 1976$).

In the mass spectra of methylates derived from saturated aldehydic acids molecular ions usually are absent. The ions of highest mass are 3 u apart, corresponding to M−31 and M−28 ions, the ion at M−28 loses a methyl radical, CH_3OH and then CO. Thus ions of mass 158, 143, 111 and 83 are produced from methyl-9-oxononanoate. The main fragments of mass 74 and of mass 87 are typical for long chain methylates – they are generated by a McLafferty rearrangement and a double hydrogen rearrangement in analogy to the reaction outlined in Fig. 6. In practice the spectra are not very informative (Figs. 60 and 61) [32,130].

The mass spectra of corresponding trimethylsilyl esters are likewise not useful for identification purposes. They show M−15 ions. Then, in the course of rearrangement reactions, H_2O or CO is lost. Peaks at m/z 73 and 75 indicate the presence of a trimethylsilyl residue, together with fragments corresponding to the loss of $(CH_3)_3SiOH$, m/z 154, M−90). The presence of a COOTMS group is further indicated by a peak at m/z 117. Fragments of mass 132 and of mass 145 result from a McLafferty resp. a ''McLafferty+13'' rearrangement (Fig. 62).

The mass spectra of the MSTFA adducts derived from methylates of ω-oxo acids (Figs. 63 and 64) are characterized by the aldehyde adducts represented by peaks at m/z 110, 134, 184, 228 and of m/z 73 and 75 as well as ions at M-15 and M-126.

Another degradation reaction is rather prominent in the mass spectra of corresponding trimethylsilylesters (Fig. 65) besides the characteristic fragments of aldehydic adducts (m/z 228, 184, 134, 110): The M−15 ion suffers a McLafferty rearrangement by loss of $CH_3N{=}C(OH)CF_3$ forming a M-142 ion (m/z 301 in Fig. 65), in this respect the degradation resembles that of trimethylsilylated MSTFA adducts of 4-hydroxy-2-alkenals (Fig. 50).

Prolonged action of aldehydic acid with MSTFA induces the generation of byproducts, e.g. of trimethylsilylated enol ethers (Fig. 66). Their spectra are characterized by an intense peak at m/z 129, for an ion derived by allylic cleavage (Fig. 67).

Methylation with diazomethane transforms partly aldehydic groups to corresponding dimethylacetals. Spectra of these compounds show a dominating ion of mass 75 generated by α cleavage and a minor one corresponding to loss of OCH_3 (m/z 201, Fig. 68) Similar fragments are observed in corresponding TMS esters.

In contrast to the above discussed spectra of adducts derived from saturated oxo acids, mass

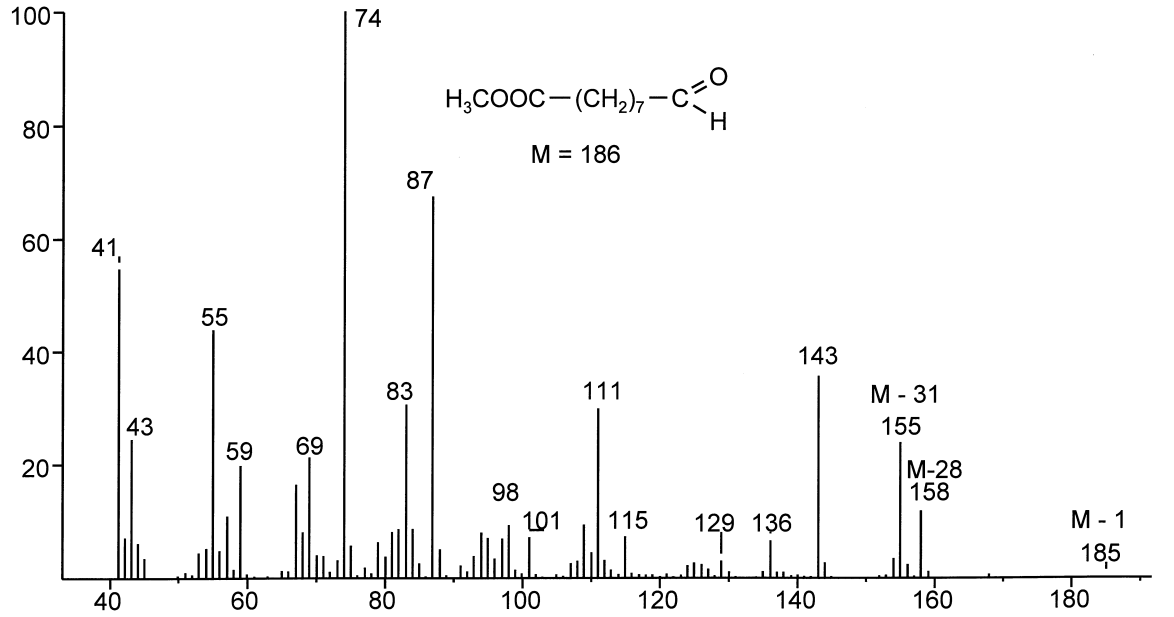


Fig. 60. Mass spectrum of methyl-9-oxononanoate ($I = 1393$), see also Kamal Eldin et al. [32] and Pfordt [130].

spectra of corresponding methoximes (Fig. 69) do show molecular ions, although the dominating fragments are the McLafferty ion and the ''McLafferty +13'' ion (m/z 73 resp. m/z 86, see Fig. 6). Additional fragments, indicative for the presence of a methoxime group, are ions at M-31. A degradation

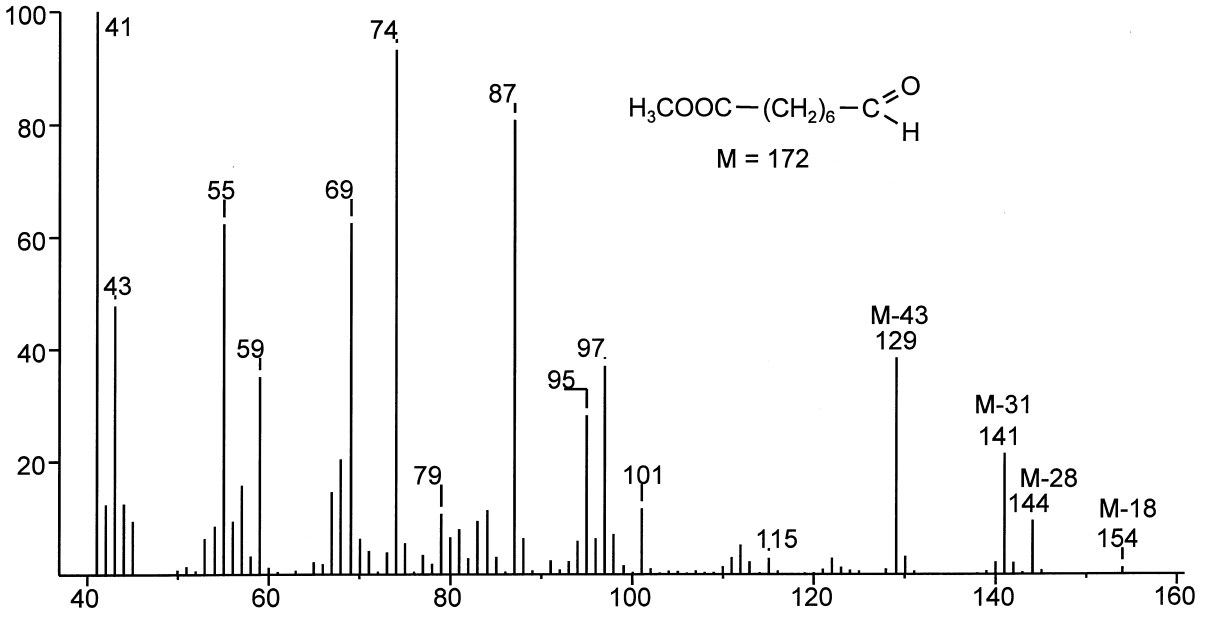


Fig. 61. Mass spectrum of methyl-8-oxooctanoate ($I = 1290$), see also Kamal Eldin et al. [32] and Pfordt [130].

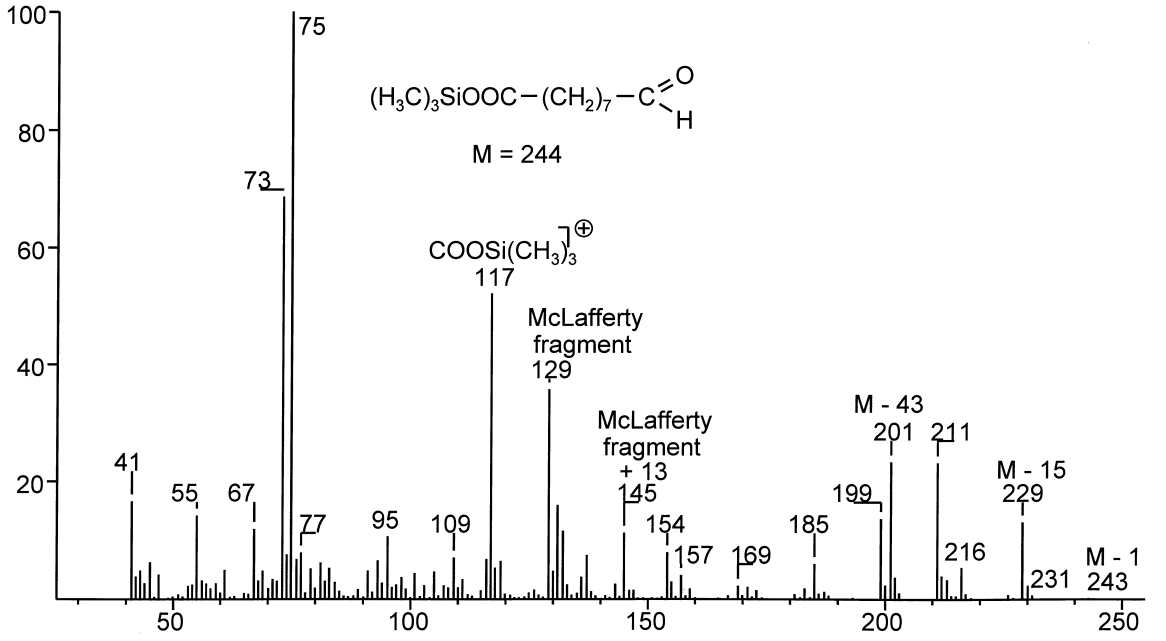


Fig. 62. Mass spectrum of trimethylsilyl-9-oxononanoate ($I = 1534$).

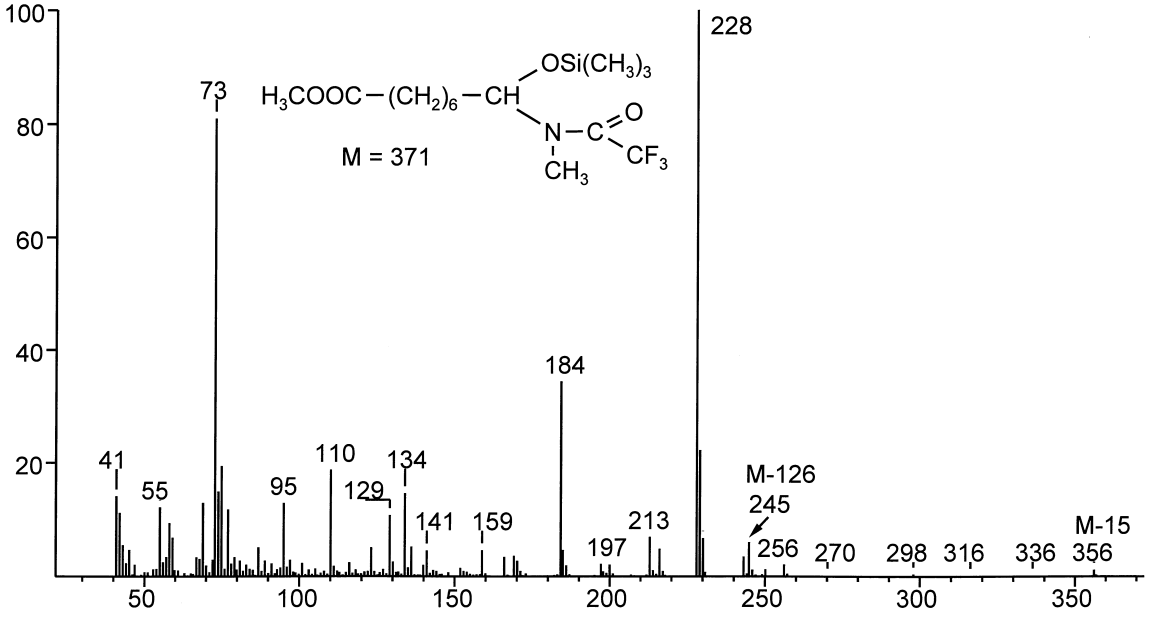


Fig. 63. Mass spectrum of the MSTFA adduct of methyl-8-oxooctanoate ($I = 1771$).

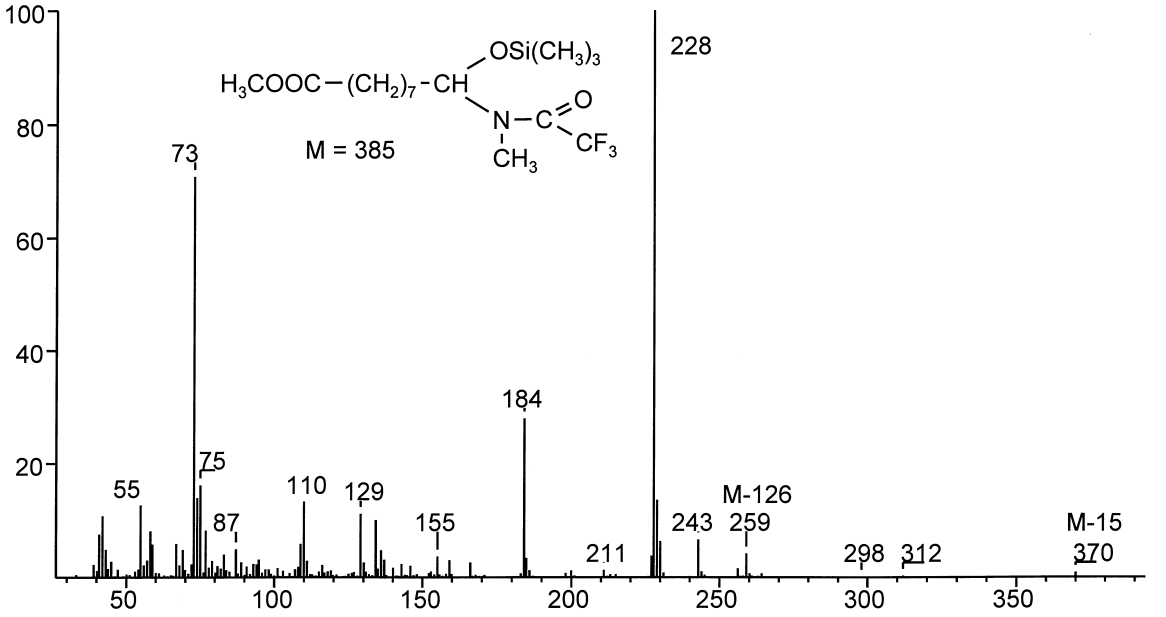


Fig. 64. Mass spectrum of the MSTFA adduct of methyl-9-oxononanoate ($I = 1874$).

starts from this ion triggered by the carbomethoxy group by loss of CH_3OH (ions at m/z 152, Fig. 69) followed by expulsion of CO (ions at m/z 124).

Very useful for structure elucidation of ω-oxo acids are pentafluorobenzyloximes: Although the spectra of the methylates do not indicate molecular

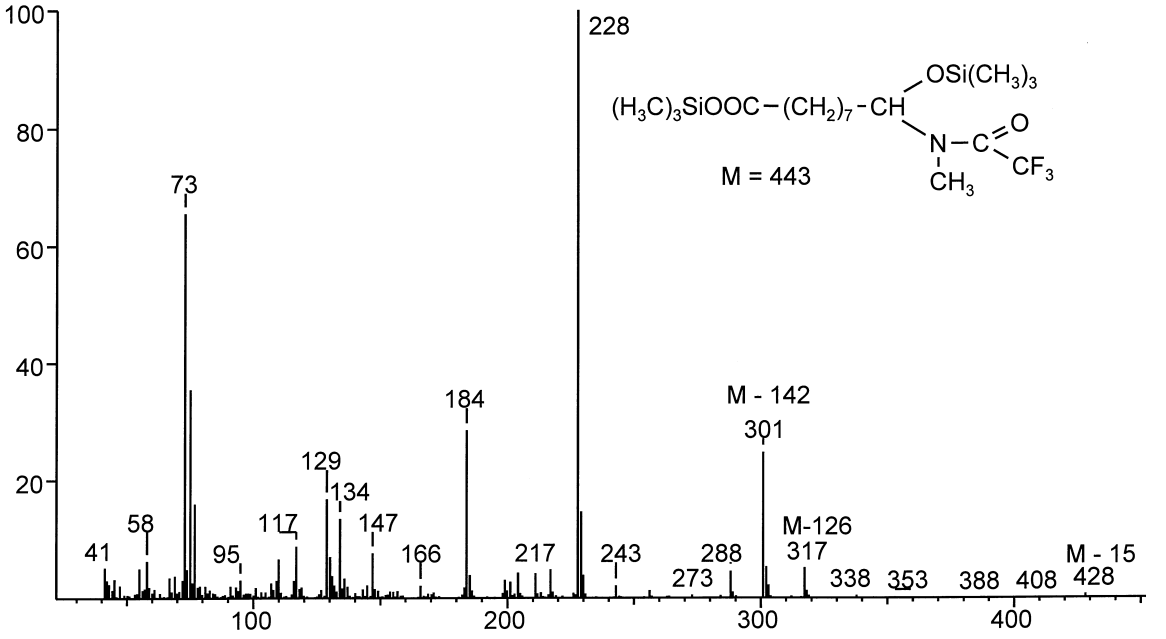


Fig. 65. Mass spectrum of the MSTFA adduct obtained from trimethylsilyl-9-oxononanoate ($I = 1986$), see also Mlakar [108].

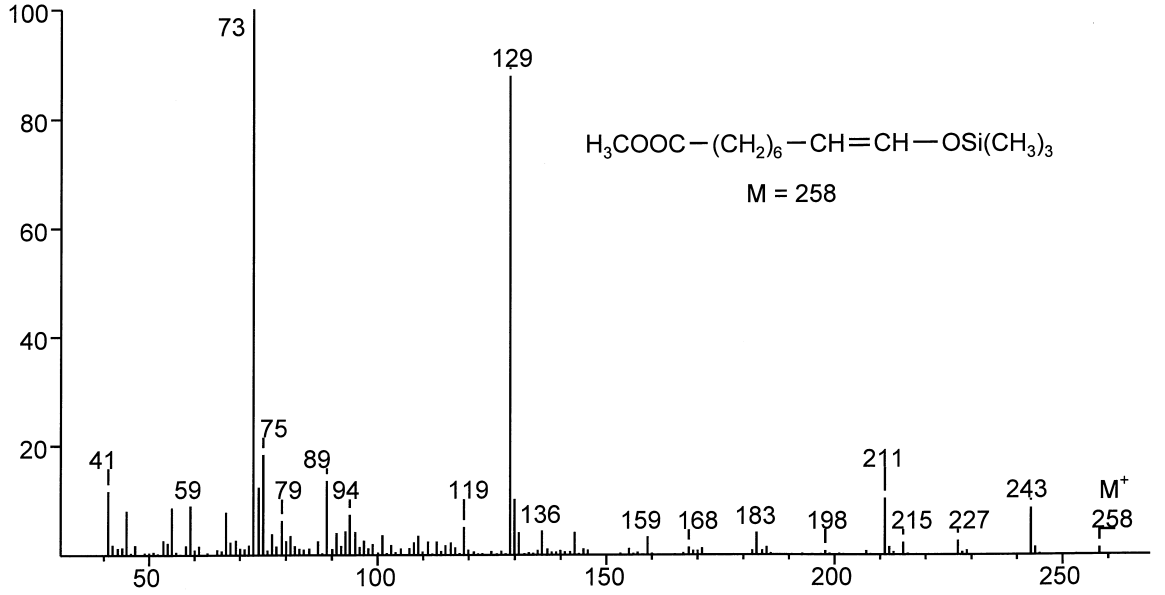


Fig. 66. Mass spectrum of the TMS enolether derived from methyl-9-oxononate ($I = 1556$), see also Vick [150].

ions the molecular mass is deduced easily by M−31 fragments (Figs. 70 and 71).

In the mass spectrum of the PFBO derivatives of the trimethylsilyl ester of 9-oxononanoic acid and trimethylsilyl-8-oxooctanoate molecular ions are of extremely low intensity (Figs. 72 and 73) M−15 ions are detectable. Nevertheless the molecular mass is readily deduced by peaks 16 u apart: These ions are generated from the M−15 ion by loss of particles of 182 u (m/z 242 in Fig. 72, m/z 228 in Fig. 73) or 198 u (m/z 226 in Fig. 72, m/z 212 in Fig. 73). These ions lose in the course of a McLafferty reaction a $CH_2=CH(OCH_3)OH$ group leading to ions of mass 168 and 152 (Fig. 72); resp. 154 and 138 (Fig. 73).

In contrast the mass spectra of di-

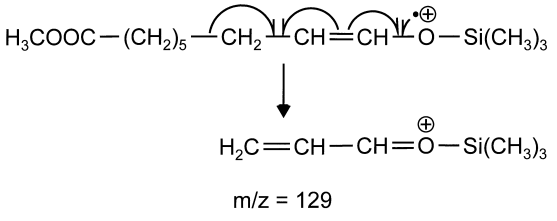


Fig. 67. Allylic cleavage of trimethylsilylenol-ethers.

methylhydrazones, derived from methyl-ω-oxo acids (Fig. 74) are characterized by abundant molecular ions. The same typical fragments (m/z 85, 86 99, 113 and 127) are observed as already discussed for the spectra of dimethylhydrazones of saturated aldehydes, which allow one to recognize the presence of a straight chain (see Figs. 24 and 27).

5.10. Unsaturated aldehydic acids

Introduction of a double bond in α,β-position to the aldehydic group of a ω-oxo acid methylate induces after ionization at the carbonyl function hydrogen abstraction from the allylic position. As a consequence mainly methanol is lost instead of $^{\cdot}OCH_3$. The M−32 fragment is further degraded by loss of CO. In addition peaks at m/z 74 and m/z 87 indicate the presence of the methylate (Fig. 75).

In the spectra of corresponding MSTFA adducts (Fig. 76) the ion corresponding to the loss of $N^{\cdot}(CH_3)$-$COCF_3$ (m/z 285) is much more dominating than in saturated aldehydic acids (Figs. 63 and 64), reflecting the enlarged mesomeric system. In addition allylic cleavage (ion m/z 268) is a main degradation reaction. Somewhat unexpected, an ion

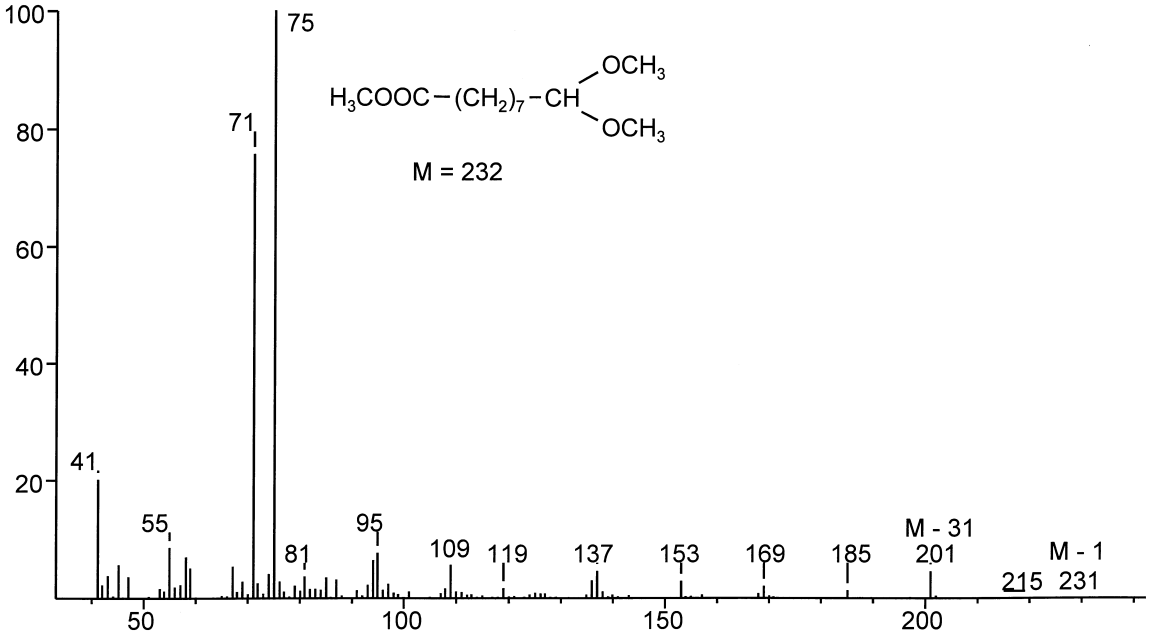


Fig. 68. Mass spectrum of the dimethylacetal derived from methyl-9-oxononanoate ($I = 1575$).

of mass 254 [loss of $(CH_2)_7$-$COOCH_3$] is formed in high abundance although this means formal cleavage of a bond adjacent to the double bond.

α,β-Unsaturated aldehydes are more prone to enolization than saturated ones when treated with MSTFA (Fig. 78):

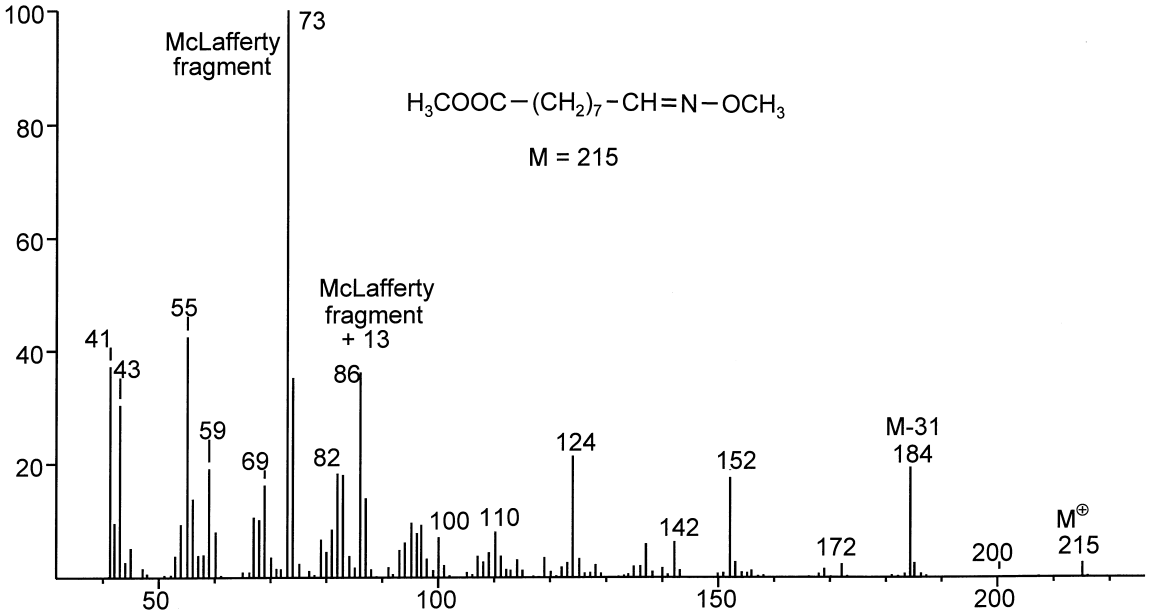


Fig. 69. Mass spectrum of the methoxime of methyl-9-oxononanoate ($I_1 = 1519$; $I_2 = 1526$).

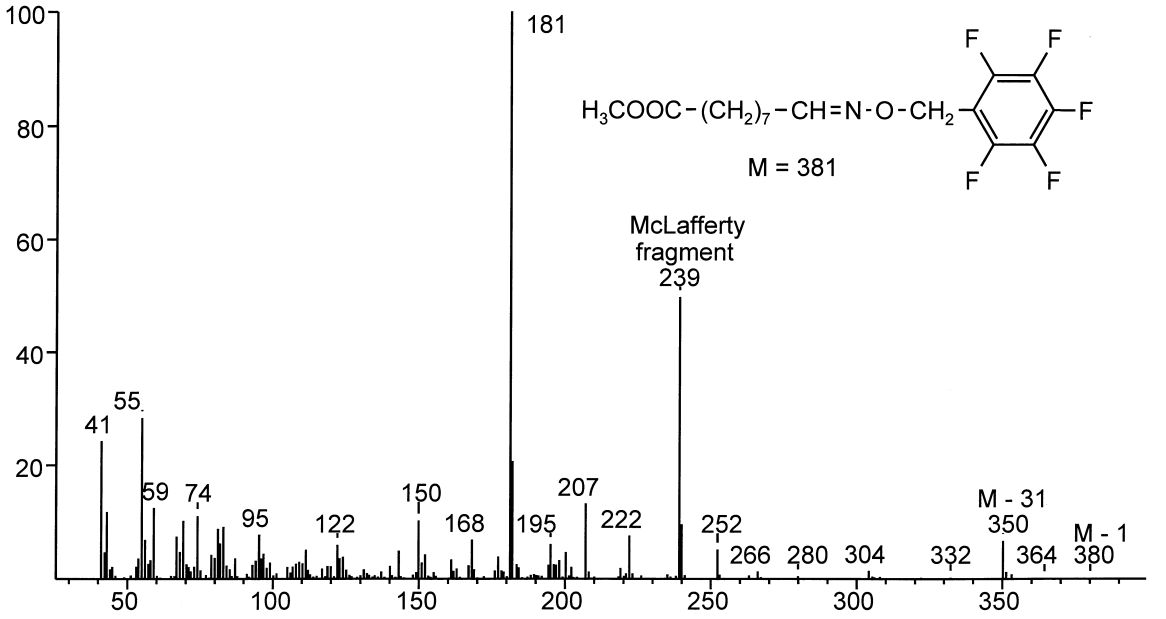


Fig. 70. Mass spectrum of the PFBO derivative of methyl-9-oxononanoate ($I_1 = 2019$; $I_2 = 2025$).

Thus a compound with a spectrum showing an intense peak at m/z 155 is generated when methyl-11-oxo-9-undecanoate is treated with MSTFA (Fig. 77).

The main degradation path of corresponding methoximes produces by a double hydrogen rearrangement (like in spectra of methoximes derived from 2-alkenals) an ion of mass 112 (compare Fig.

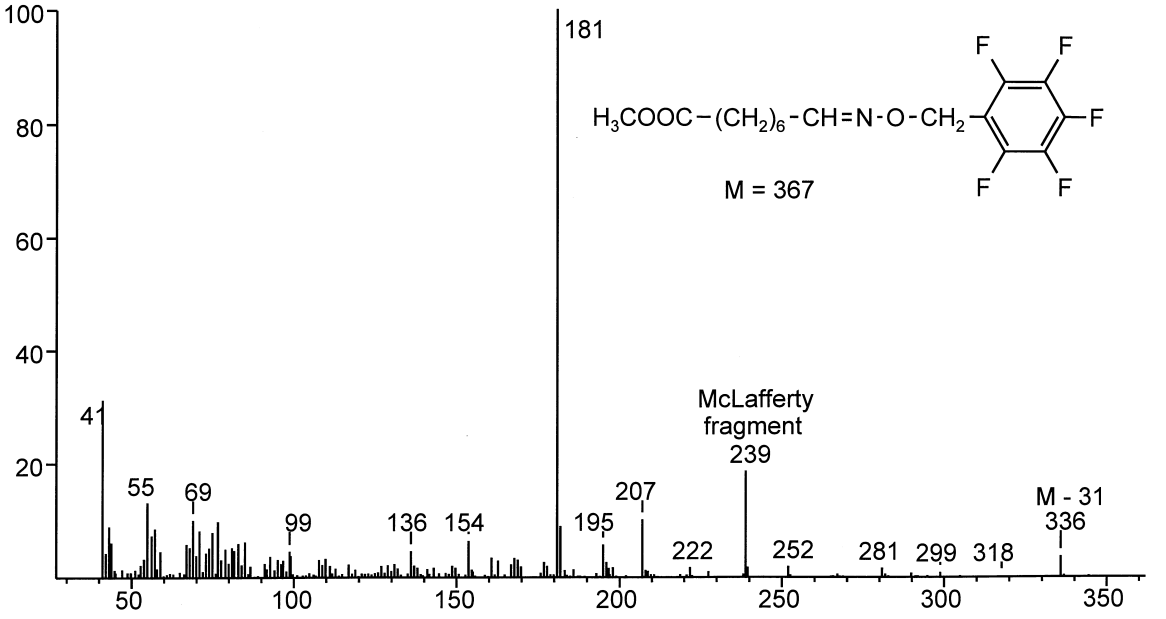


Fig. 71. Mass spectrum of the PFBO derivative of methyl-8-oxooctanoate ($I_1 = 1926$; $I_2 = 1931$).

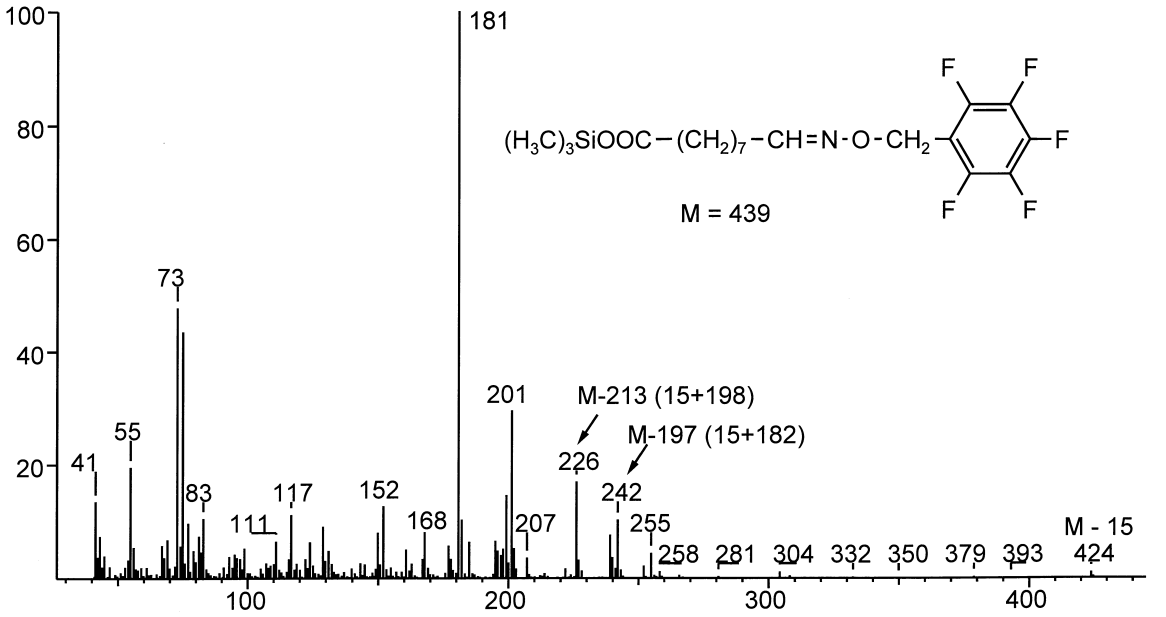


Fig. 72. Mass spectrum of the PFBO derivative of trimethylsilyl-9-oxononanoate ($I=2160$).

29). Besides M-63 ions indicate loss of $^{\cdot}OCH_3$ and of CH_3OH, typical for methoximes derived from methylates (Fig. 79).

The mass spectra of the PFBO derivatives of α,β-unsaturated aldehydes show rather abundant molecular ions. In addition peaks are observed which correspond to loss of $^{\cdot}OCH_3$ (m/z 376),181 u (m/z 226) and 197 u (m/z 210), (Fig. 80).

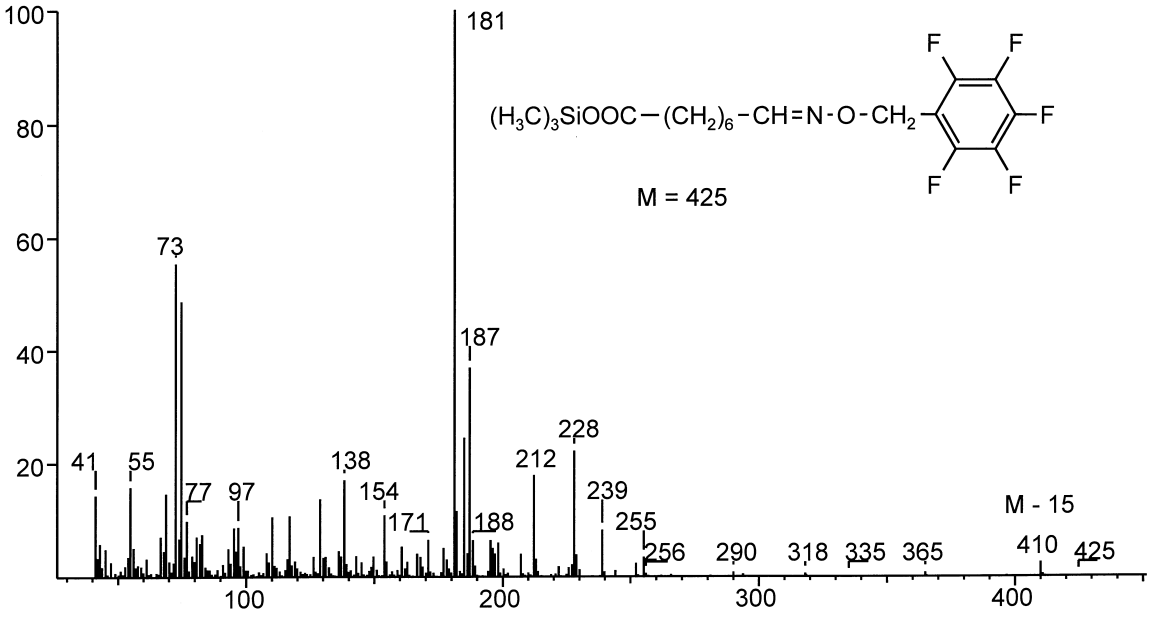


Fig. 73. Mass spectrum of the PFBO derivative of trimethylsilyl-8-oxooctanoate ($I=2062$).

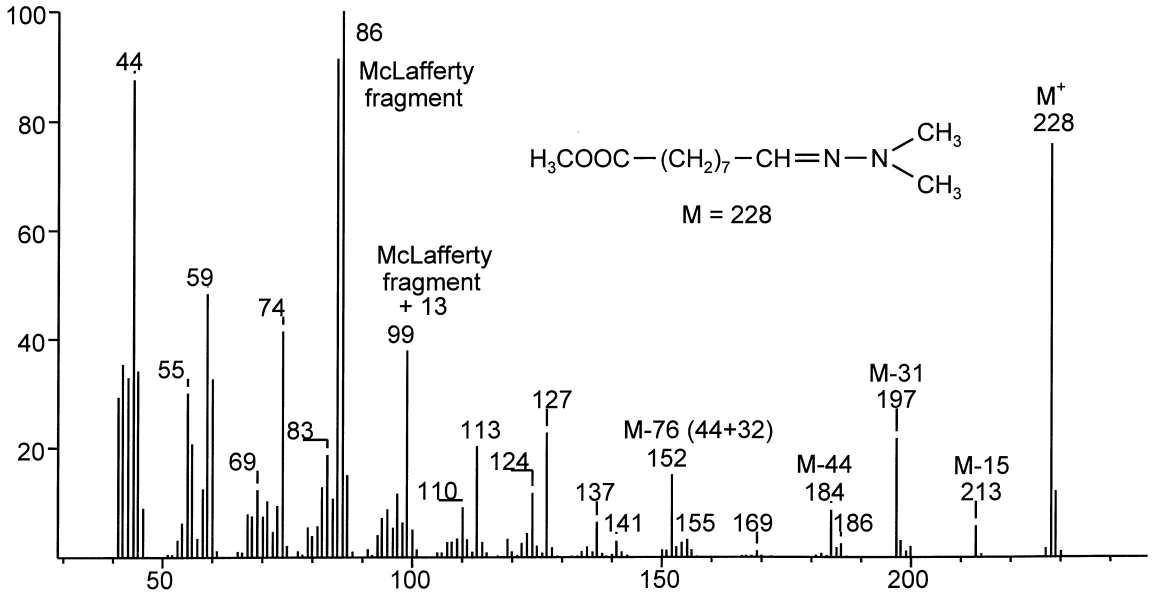


Fig. 74. Mass spectrum of the dimethylhydrazine derivative of methyl-9-oxononanoate ($I = 1661$).

The mass spectra of dimethylhydrazones of α,β-unsaturated aldehydic acids show prominent molecular ions and typical ions of mass 111, derived by allylic cleavage (Fig. 81).

Since 2,4-dinitrophenylhydrazones of oxo acid esters are of rather low volatility and since PBFO derivatives are readily available and their mass

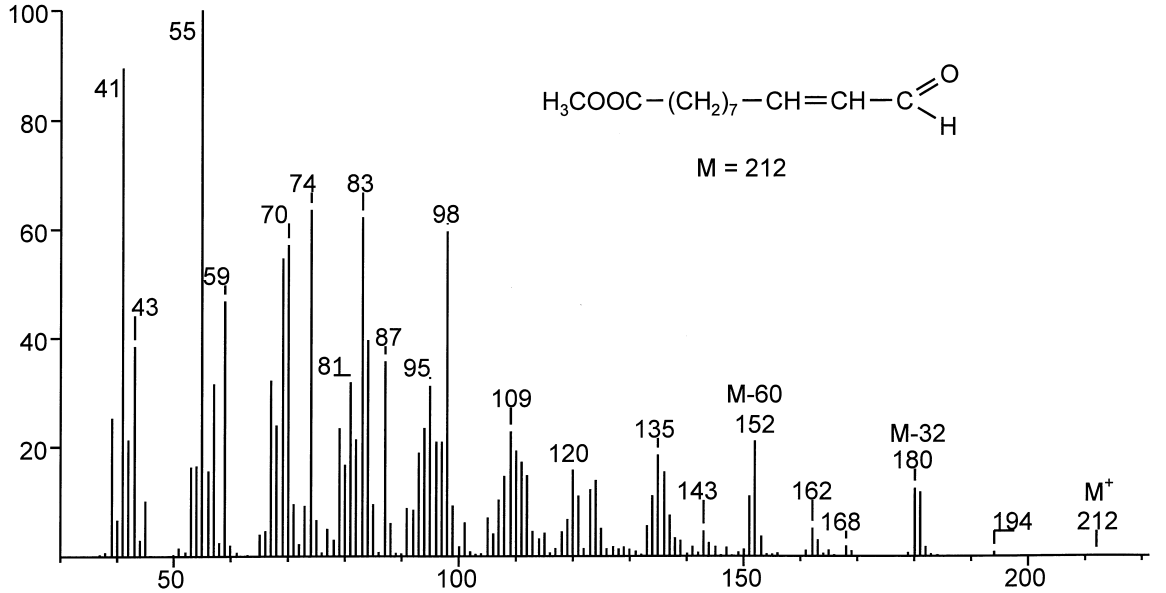


Fig. 75. Mass spectrum of methyl-11-oxo-9-undecenoate ($I = 1650$), see Kamal-Eldin [32].

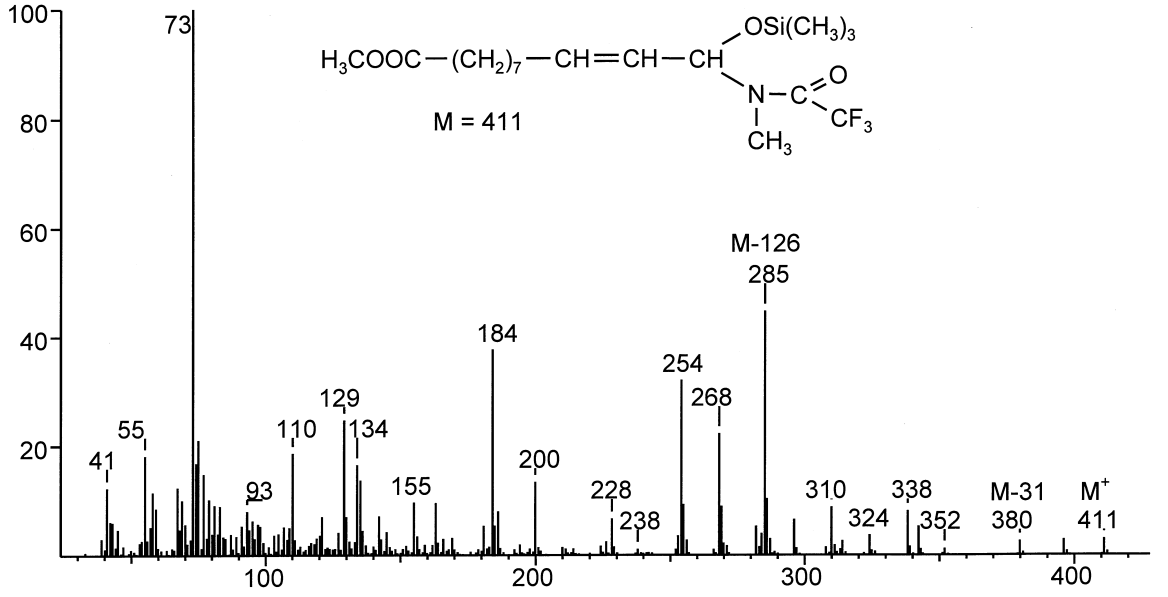


Fig. 76. Mass spectrum of the MSTFA adduct obtained from methyl-11-oxo-9-undecenoate ($I = 2047$).

spectra are sufficiently informative, spectra of 2,4-dinitrophenylhydrazones are not reproduced.

5.11. α,β,γ,δ-Unsaturated aldehydic acids

The tendency for Michael addition reactions increases with an increasing number of double bonds in conjunction to the aldehyde group. Thus for instance diazomethane reacts with unsaturated carbonyl systems by addition to the double bond. Therefore the reaction time should never exceed 1–2 min [50]. If such reactions might be expected,

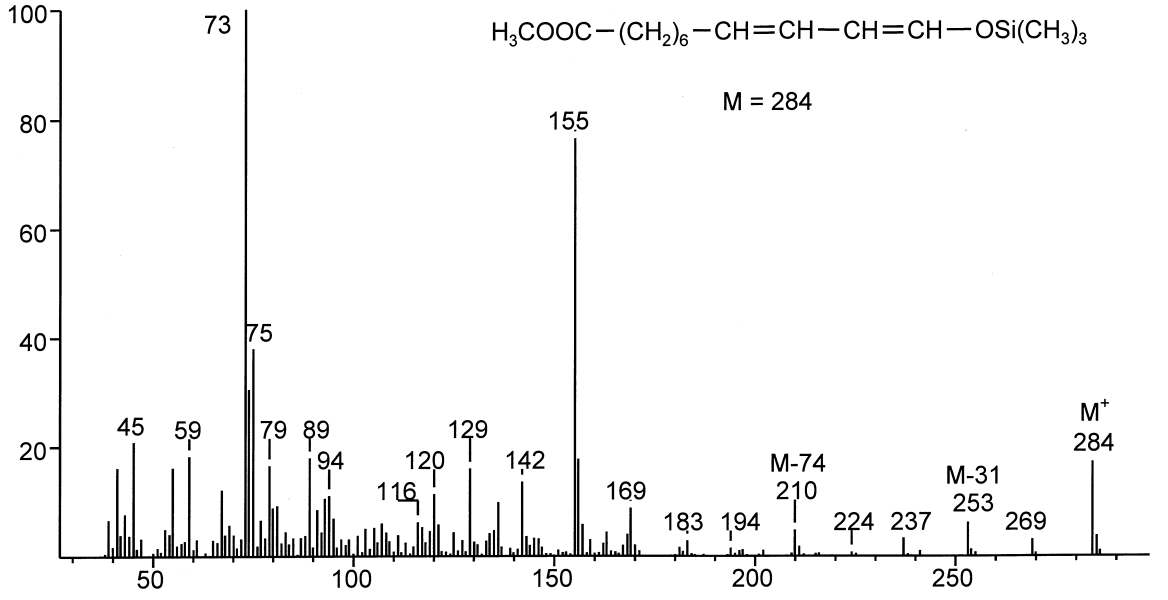


Fig. 77. Mass spectrum of methyl-11-trimethylsilyloxy-8,10-undecadienoate ($I_1 = 1826$; $I_2 = 1859$).

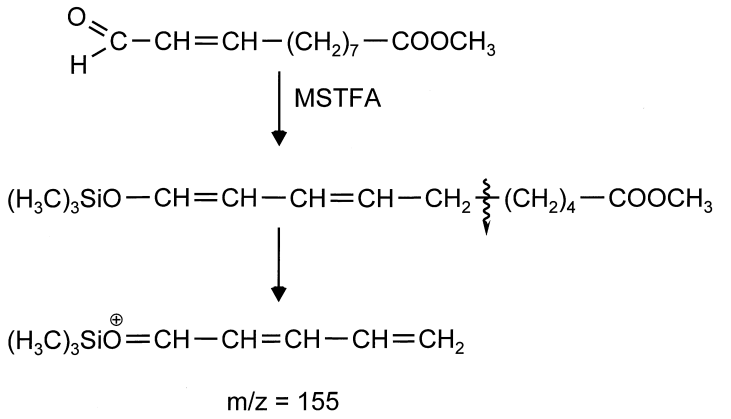


Fig. 78. Enolization of α,β-unsaturated aldehydic compounds by treatment with MSTFA causing allylic cleavage.

trimethylsilylation of the carboxylic group is preferable.

In mass spectra of α,β,γ,δ-unsaturated aldehydic acids, obtained by LPO processes, the tendency to generate molecular ions in respect to α,β-unsaturated aldehydic acids is even enhanced. The mass spectrometric degradation reactions of α,β,γ,δ-unsaturated aldehyde acid methylates and their derivatives are triggered by the unsaturated conjugated carbonyl system and resemble therefore those of 2,4-dienals (compare for Figs. 36 and 37). Thus a main fragment of mass 81 is observed in the spectrum of methyl-13-oxo-9-[*cis*,11-*trans*-tridecadienoate (Fig. 82) [131,132] corresponding to the pyrylium ion (see Fig. 35).

The tendency to generate onium ions is also typical for aldehyde adducts of α,β,γ,δ-unsaturated aldehydes: The mass spectra of the PFBO derivatives [97] and the MSTFA adducts of α,β,γ,δ-aldehydic acids are highly informative: Thus the mass spectrum

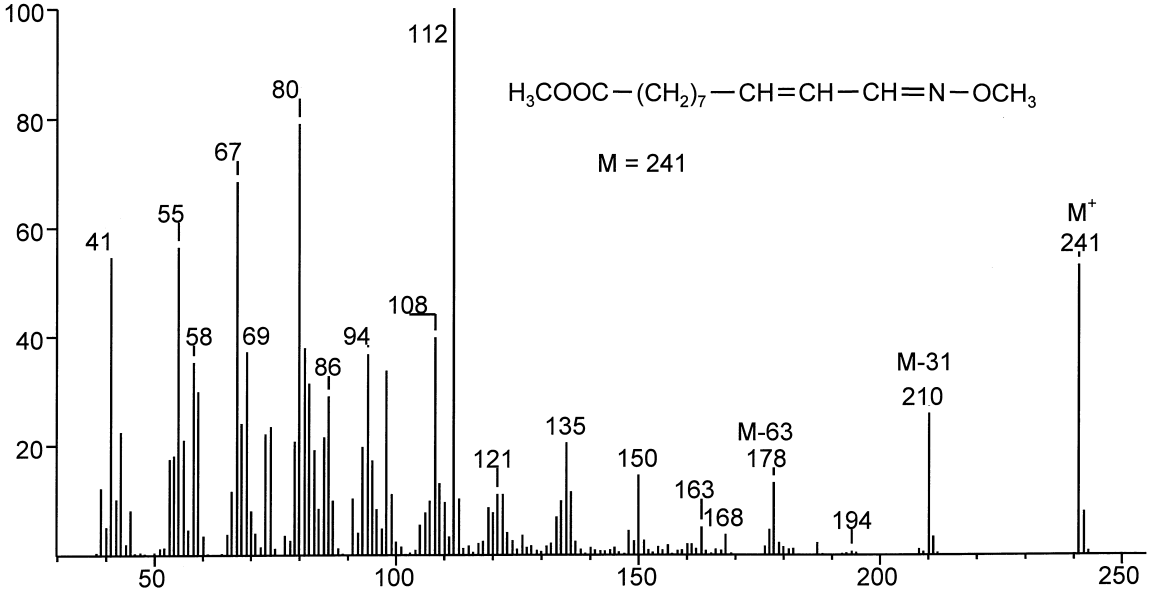


Fig. 79. Mass spectrum of the methoxime derivative of methyl-11-oxo-9-undecenoate ($I_1 = 1759$, $I_2 = 1766$).

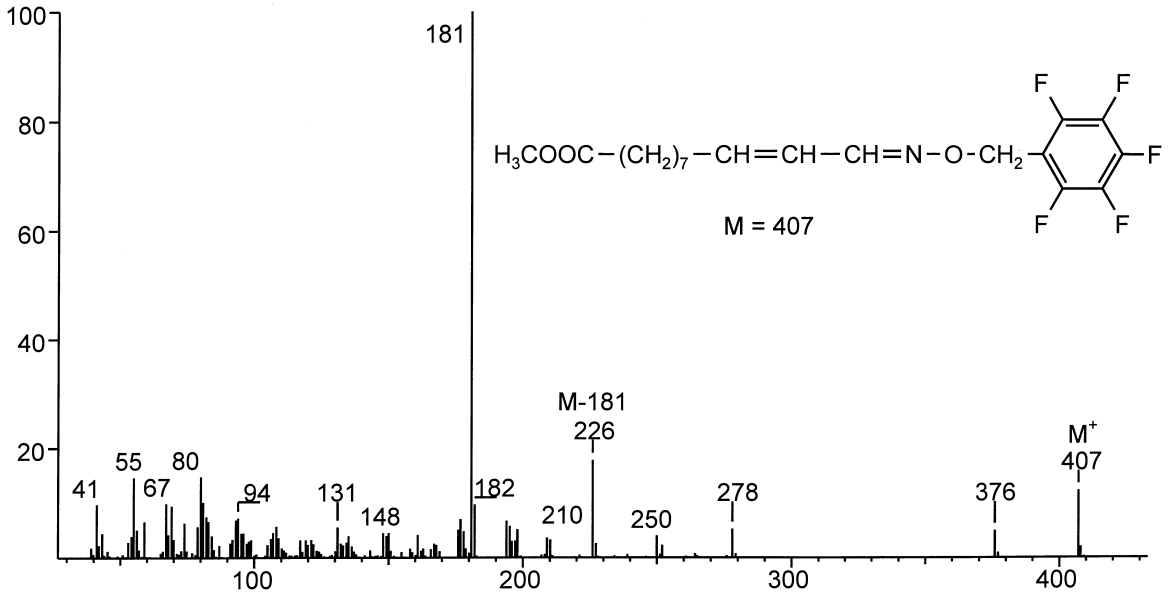


Fig. 80. Mass spectrum of the PFBO adduct of methyl-11-oxo-9-undecenoate ($I_1 = 2283$, $I_2 = 2316$).

of the PFBO derivative of methyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate (Fig. 83) shows a prominent molecular ion peak. A fragment M-31 and one (admittedly of low abundance) at m/z 59 are indicative for the presence of a $COOCH_3$ group. The ions of mass 181, M-181 (m/z 252) and M-197 (m/z 236) are typical fragment ions of unsaturated PFBO aldehyde derivatives. The ion of mass 276 is gener-

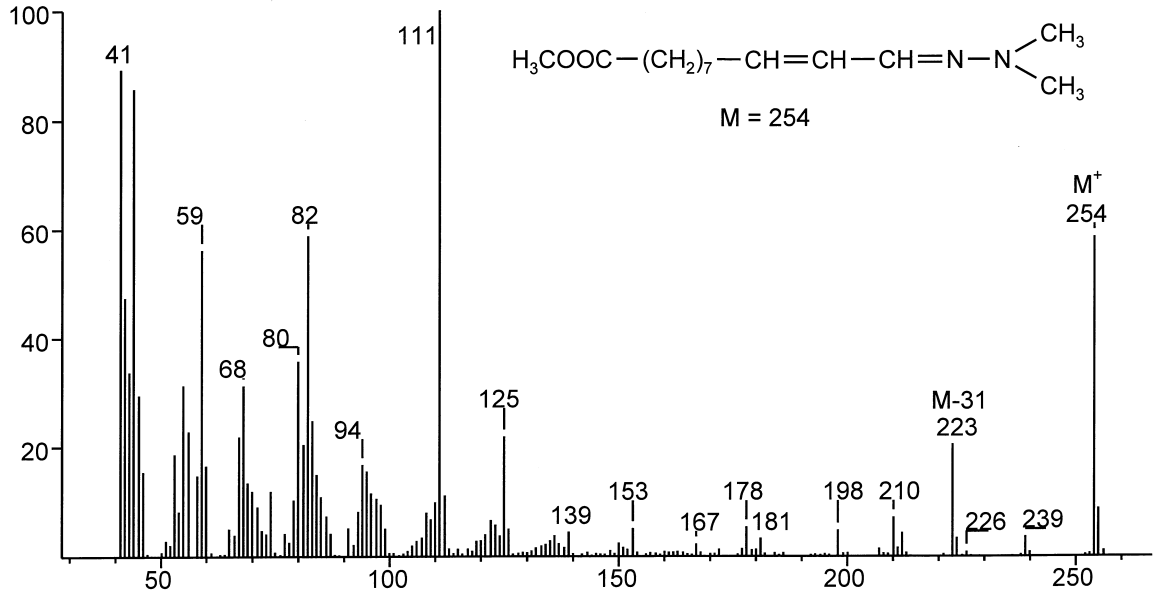


Fig. 81. Mass spectrum of the dimethylhydrazine derivative of methyl-11-oxo-9-undecenoate ($I = 1952$).

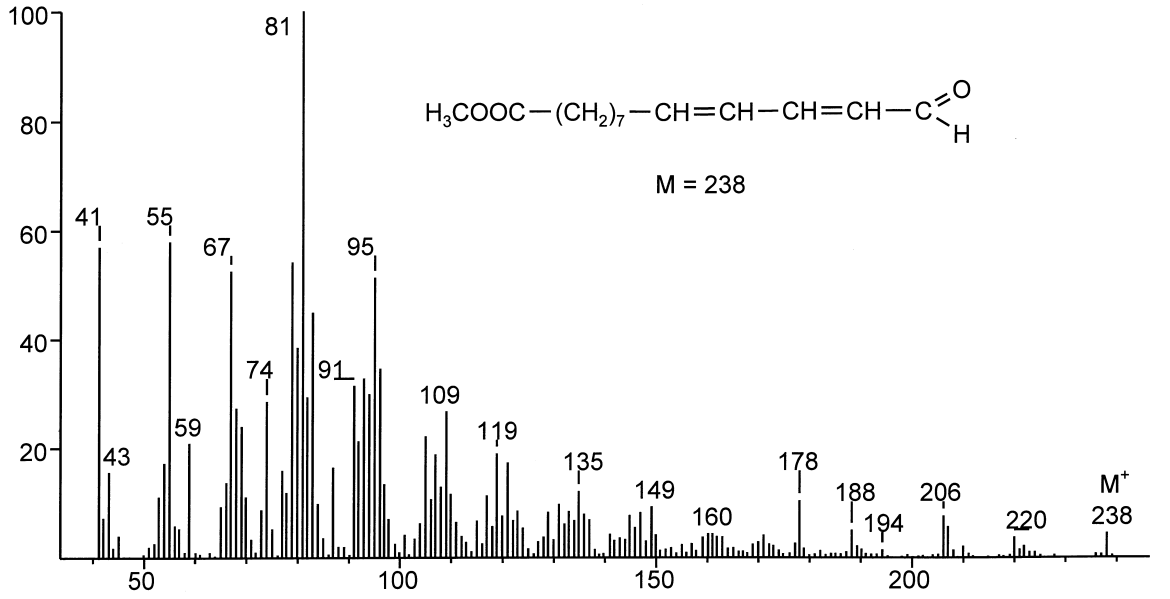


Fig. 82. Mass spectrum of methyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate ($I=1880$), see also Salch [132], Vick [132], Gardner [133] and Tahara [139].

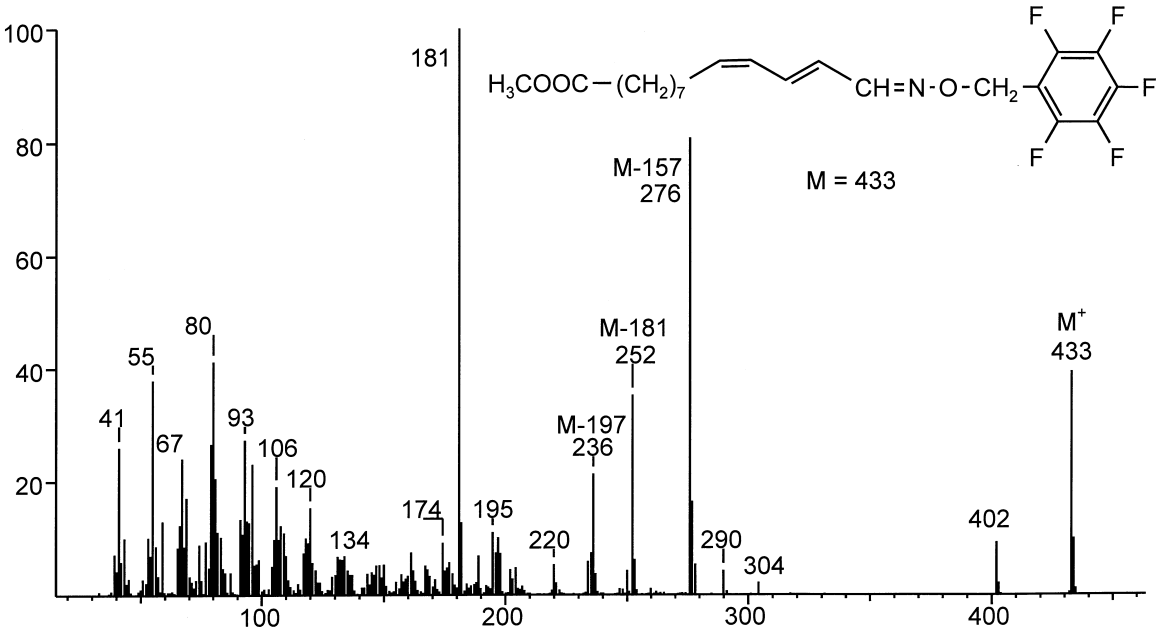


Fig. 83. Mass spectrum of the PFBO derivative of the methyl-13-oxo-9-*cis*,11-*trans*-tridecadienonate ($I=2548$).

ated analogously to the reaction outlined in Fig. 38. This ion loses then one molecule of pentafluorobenzaldehyde to generate the ion of mass 80, both ions together are a strong hint for the presence of an α,β,γ,δ-unsaturated aldehydic partial structure in the original compound.

Fig. 84 shows the mass spectrum of the MSTFA adduct obtained from methyl 13-oxo-9-*cis*,11-*trans*-tridecadienoate. It is characterized by a key ion of mass 308, produced in analogy to Fig. 41. In addition, the typical ions of MSTFA adducts are recognized: m/z 110, m/z 134, m/z 184, M−126 (m/z 311), M−69 (M−CF_3, m/z 368) and M−97 (M−$COCF_3$, m/z 340). Similar degradation reactions are observed in the spectrum of the corresponding trimethylsilylester (Fig. 85). In addition the typical ions for MSTFA adducts M−127 (m/z 368) and M−142 (m/z 353) are rather prominent, characteristic for MSTFA adducts of trimethylsilylesters, as outlined above.

Thus there is a remarkable difference in the fragmentation behaviour of aldehyde oxime derivatives and corresponding MSTFA adducts of α,β,γ,δ-unsaturated aldehydes, recognized by a comparison of their spectra: In the oxime derivatives cleavage by formation of pyridinium ions prevails while the most abundant products are generated from MSTFA adducts by allylic cleavage (ion m/z 308, compare Figs. 83 and 84). As a consequence the generation of different types of derivatives is especially useful for structure elucidation of these aldehydic compounds.

5.12. Hydroxy-oxo acids

Aldehydic acids with an OH group in α-position to the aldehydic function are generated in similar way from 9-HPODE as α-hydroxyaldehydes from 13-HPODE.

The investigation of aldehydic acids with an additional hydroxy function by GC–MS requires after derivatization of the carbonyl function methylation of the carboxylic group and finally protection of the hydroxy function by trimethylsilylation, e.g. by treatment with MSTFA. Alternatively the compound mixture is first reacted with the aldehyde trapping reagent and then derivatized with MSTFA to obtain in one step trimethylsilylation of the carboxylic group and the hydroxy group.

Main fragmentation routes are similar to corresponding saturated aldehydes (see above), thus for instance the methoxime derivative of 9-trimethyl-

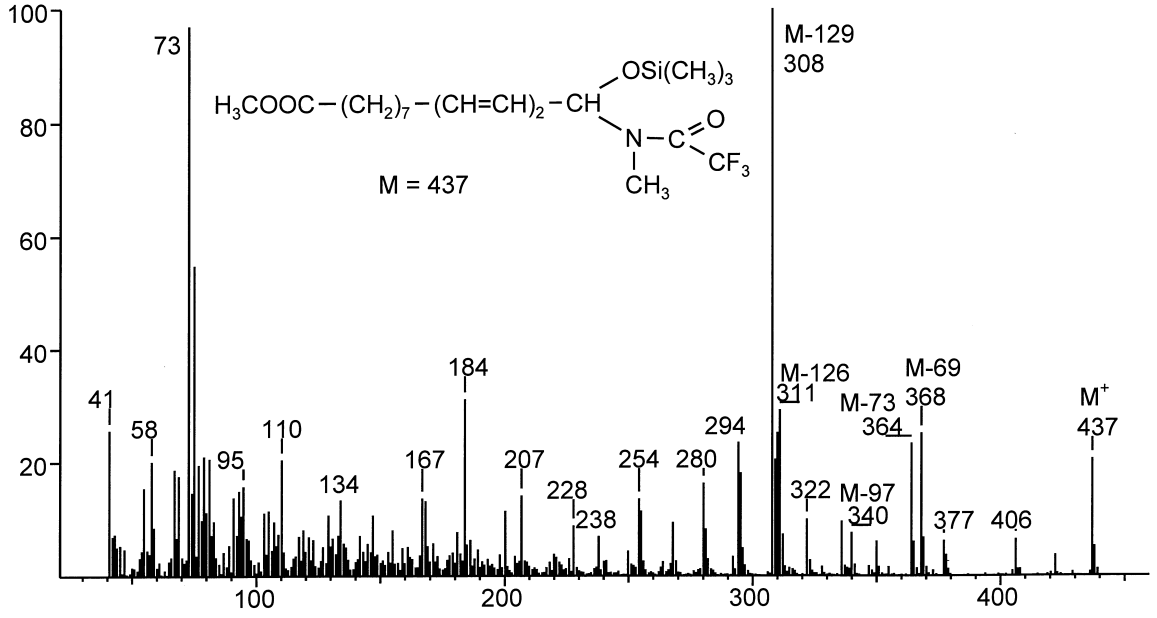


Fig. 84. Mass spectrum of the MSTFA adduct of methyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate (I=2221).

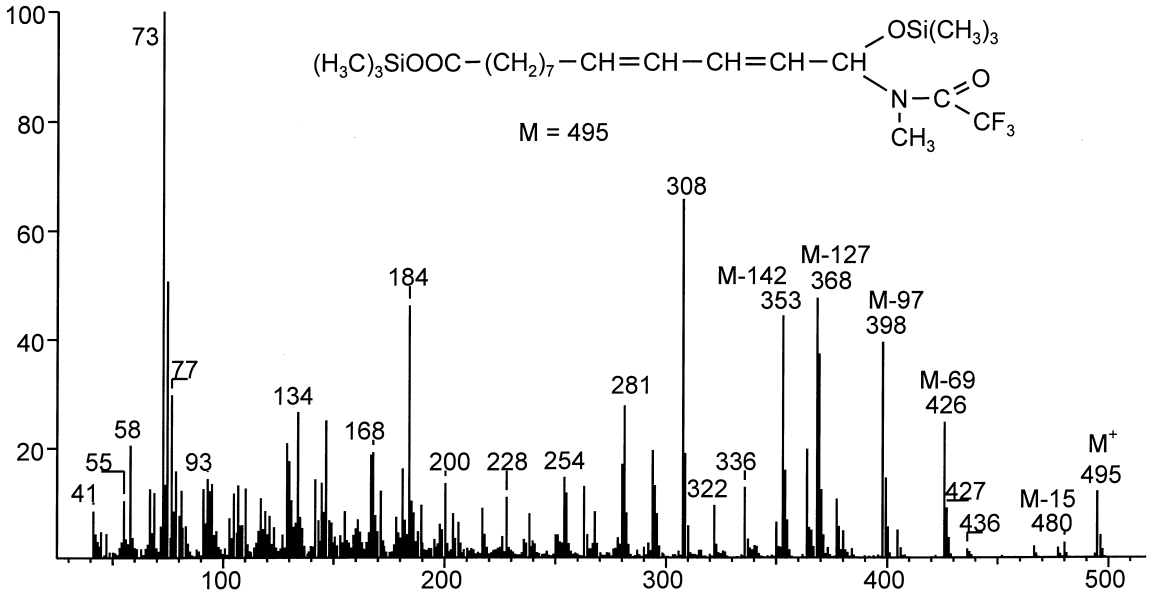


Fig. 85. Mass spectrum of the MSTFA adduct of trimethylsilyl-13-oxo-9-*cis*,11-*trans*-tridecadienoate ($I = 2349$).

silyloxy-10-oxodecanoic acid (Fig. 86) suffers in the mass spectrometer mainly cleavage in α-position to the trimethylsiloxy group to generate a fragment m/z 160. Further typical ions are formed by expulsion of $^{\cdot}OCH_3$, (m/z 286) and methanol and a methyl radical (m/z 270). The carboxylic group is indicated by an ion of mass 59 (Fig. 86).

The mass spectrum of the trimethylsilylated PFBO derivative of methyl-9-hydroxy-10-oxodecanoate (Fig. 88) is characterized – besides the ion of mass

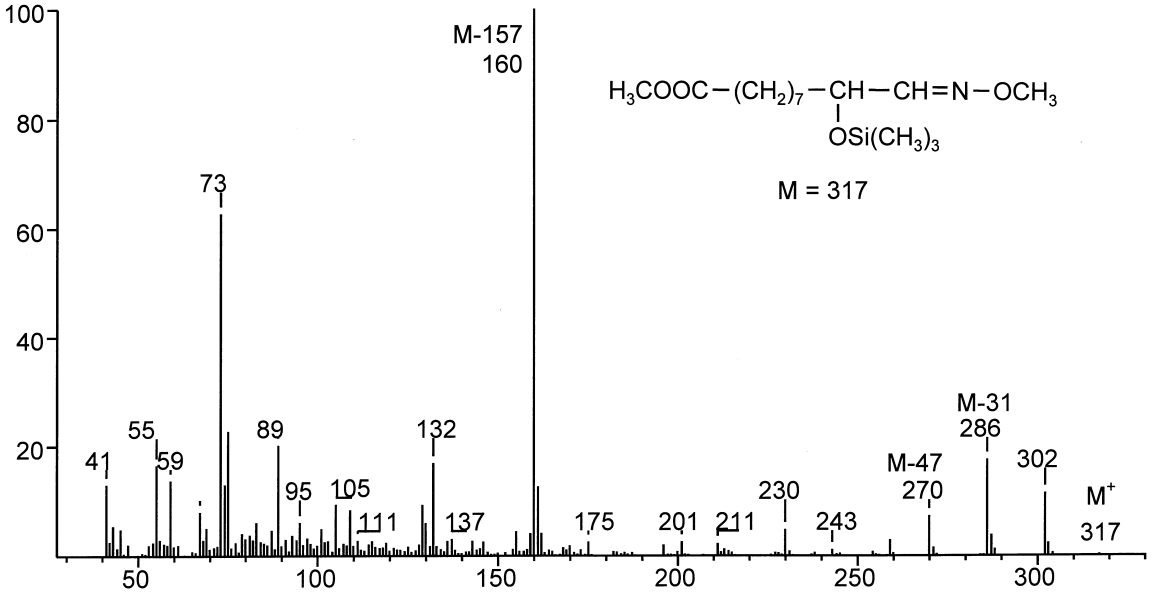


Fig. 86. Mass spectrum of the methoxime derived from methyl-9-trimethylsilyloxy-10-oxodecanoate* ($I_1 = 1796$*; $I_2 = 1815$*).

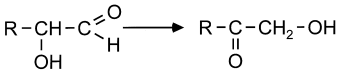


Fig. 87. Rearrangement of α-hydroxyaldehydes to 1-hydroxy-2-alkanones.

181 – by an abundant ion indicating α-cleavage (m/z 326). This ion loses then CO to generate the ion of mass 298. Two additional peaks at m/z 270 (not very intense) and m/z 286 correspond to the loss of particles with 181 and 197 u, typical reactions of PFBO derivatives of α,β-unsaturated aldehydes. These ions allow the calculation of the molecular mass which is additionally confirmed by the presence of M−15 and M−31 ions.

The deduction of cleavage processes is considerably facilitated if spectra of homologues are available, thus for instance, by degradation reactions of hydroperoxides derived from PUFAs the lower homologues of acidic degradation products are obtained, admittedly often in low yield only. The lower homologue of 9-hydroxy-10-oxodecanoic acid, 8-hydroxy-9-oxononanoic acid, undergoes the same degradation reactions as its higher homologues indicated by a shift of corresponding ions (Figs. 88 and 89).

α-Hydroxyaldehydes suffer easily rearrangement to 2-oxo-1-alkanols (Fig. 87) [134,135].

The rearranged products accompany the α-hydroxyaldehydes. The isomeric PFBO derivatives show very different spectra: Besides the ion of m/z 181, a main peak is found at M−15 (m/z 468) other prominent ions are formed by loss of the pentafluorobenzyl residue (M−181, m/z 302) and by loss of the pentafluorobenzyloxy radical (M−197, m/z 286). These ions lose then in a McLafferty type reaction the side chain to form the fragments of mass 124 and of mass 152. The presence of the CH_2-OTMS group is recognized by a prominent peak at m/z 103 (Fig. 90).

5.13. α,β-Unsaturated aldehydic acids with a hydroxy function in position ω-4

Aldehydic acids containing the structural element OHC-CH=CH-CH(OTMS)- are characterized in their spectra (Fig. 91) by an ion of m/z 157, as outlined above (see also Fig. 49).

Main cleavage products in the mass spectrum of the MSTFA adduct of methyl-9-trimethylsilyloxy-

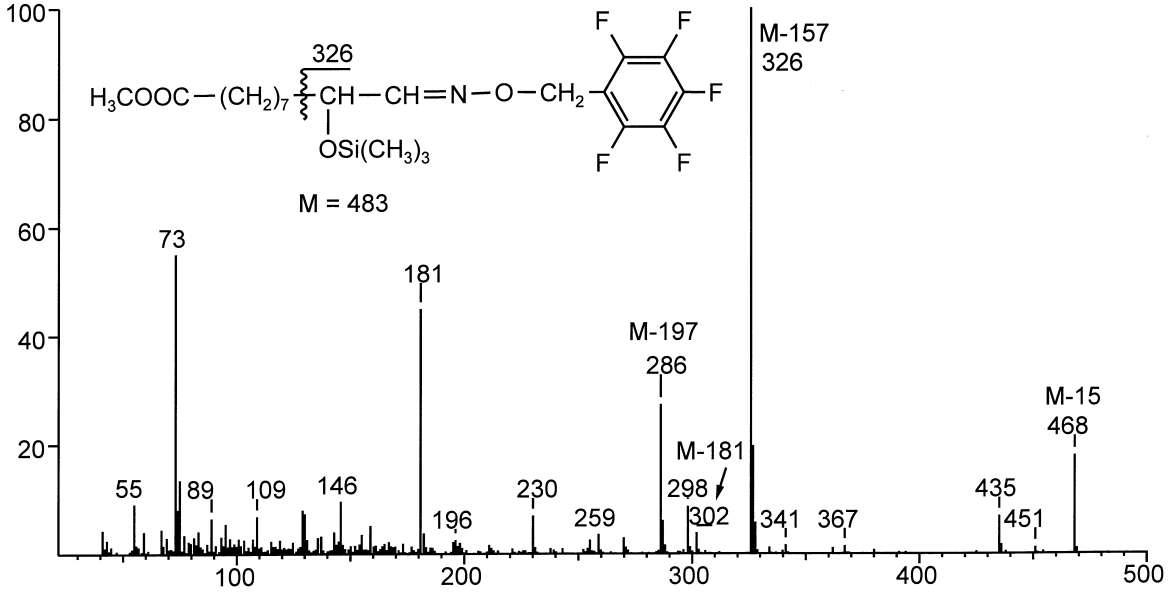


Fig. 88. Mass spectrum of the PFBO derivative generated from methyl-9-trimethylsilyloxy-10-oxodecanoate*, see also Mlakar [108] (for COO-TMS derivatives and MSTFA adduct) (I=2293*).

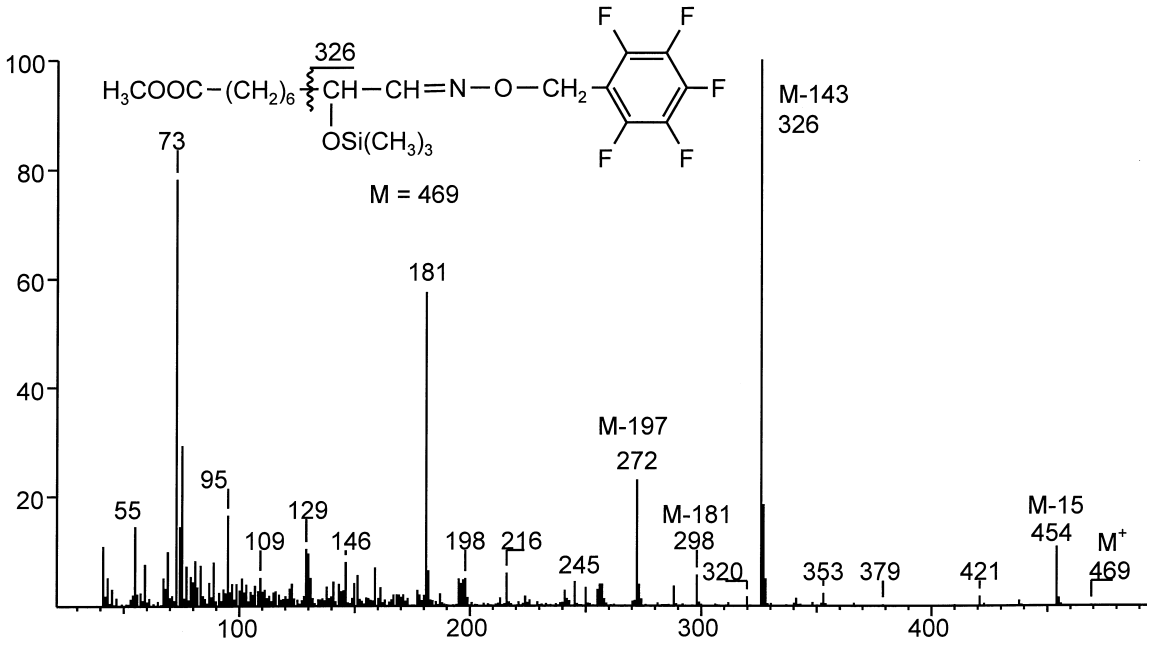


Fig. 89. Mass spectrum of the PFBO derivative of methyl-8-trimethylsilyloxy-9-oxononanoate*, see also Loidl-Stahlhofen [81] (as *tert.*-butyl-dimethylsilyl-ether) ($I = 2237$*).

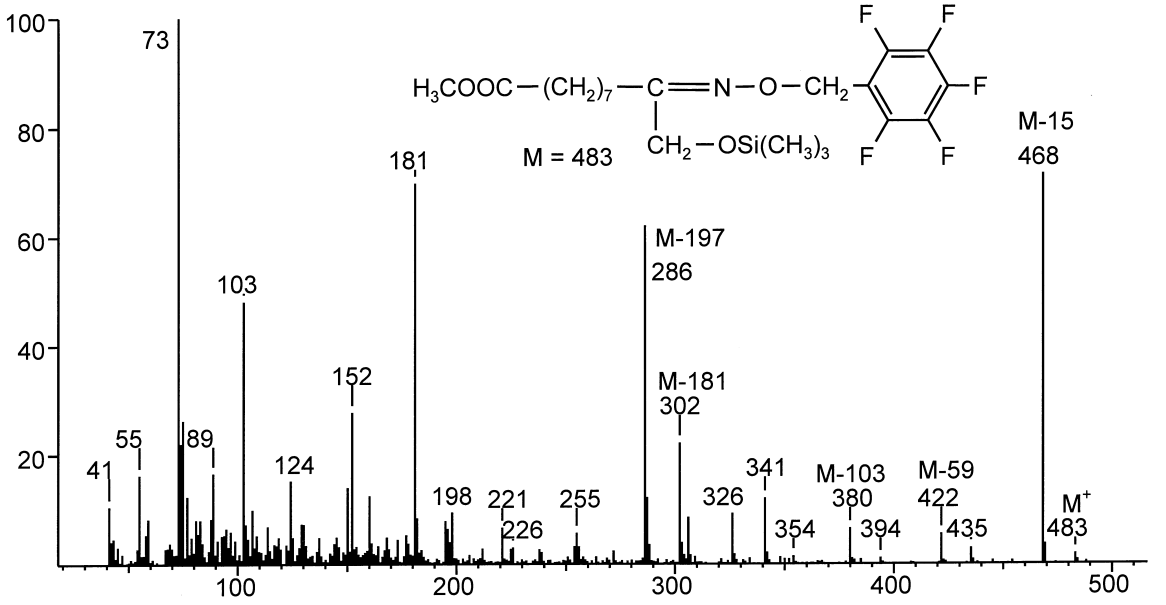


Fig. 90. Mass spectrum of the PFBO derivative of methyl-10-trimethylsilyloxy-9-oxodecanoate* ($I = 2325$*).

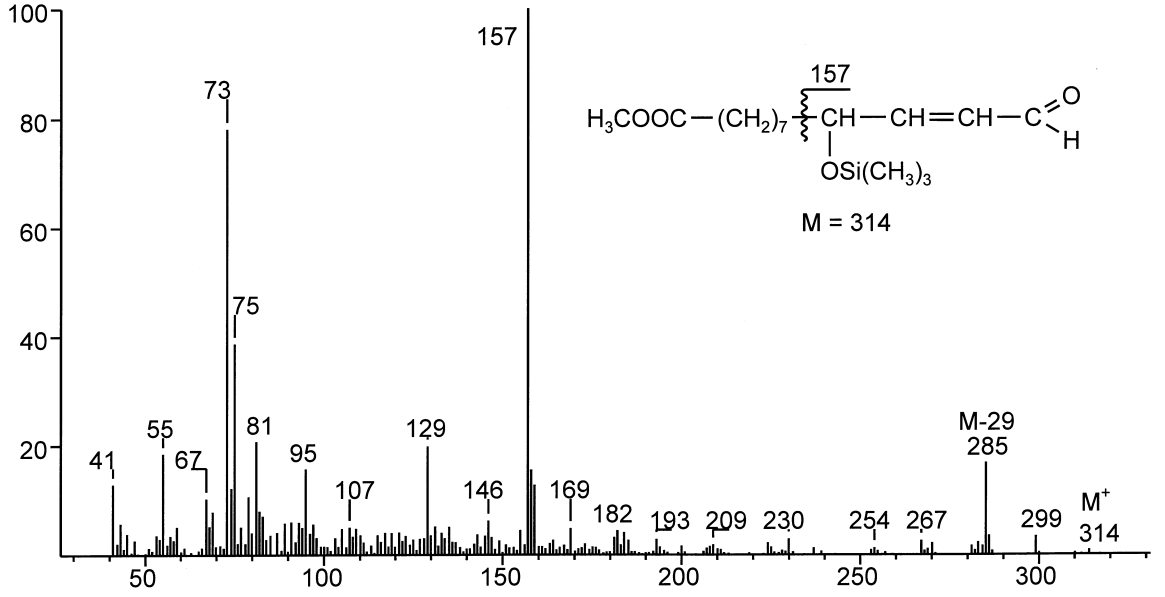


Fig. 91. Mass spectrum of the methyl-9-trimethylsilyloxy-12-oxo-10-dodecenoate* ($I = 1983$*).

12-oxo-10-dodecenoate (Fig. 92) are derived by loss of a particle with 126 u (m/z 285). In addition an allylic cleavage product (m/z 268) is formed preferentially. Somewhat unexpected is the generation of the ion of mass 254 which is formally produced by cleavage of the C-9/C-10 bond (Fig. 92).

The mass spectrum of the methoxime derivative of methyl-9-trimethylsilyloxy-12-oxo-10-dodecenoate

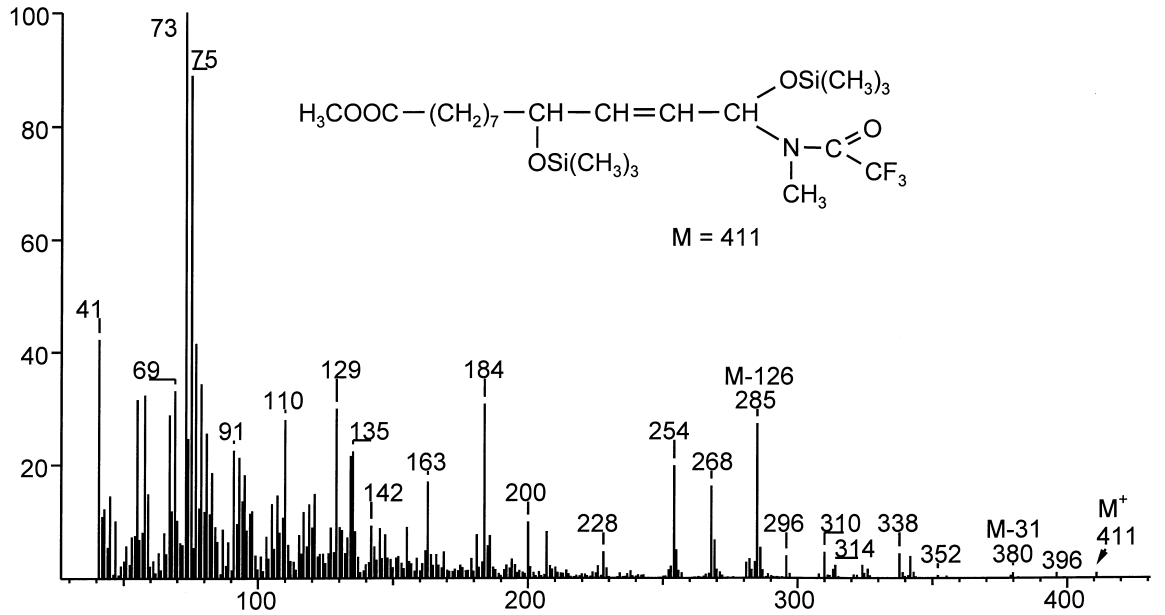


Fig. 92. Mass spectrum of the MSTFA adduct of methyl-9-trimethylsilyloxy-12-oxo-10-dodecenoate* ($I_1 = 2248$*; $I_2 = 2254$*).

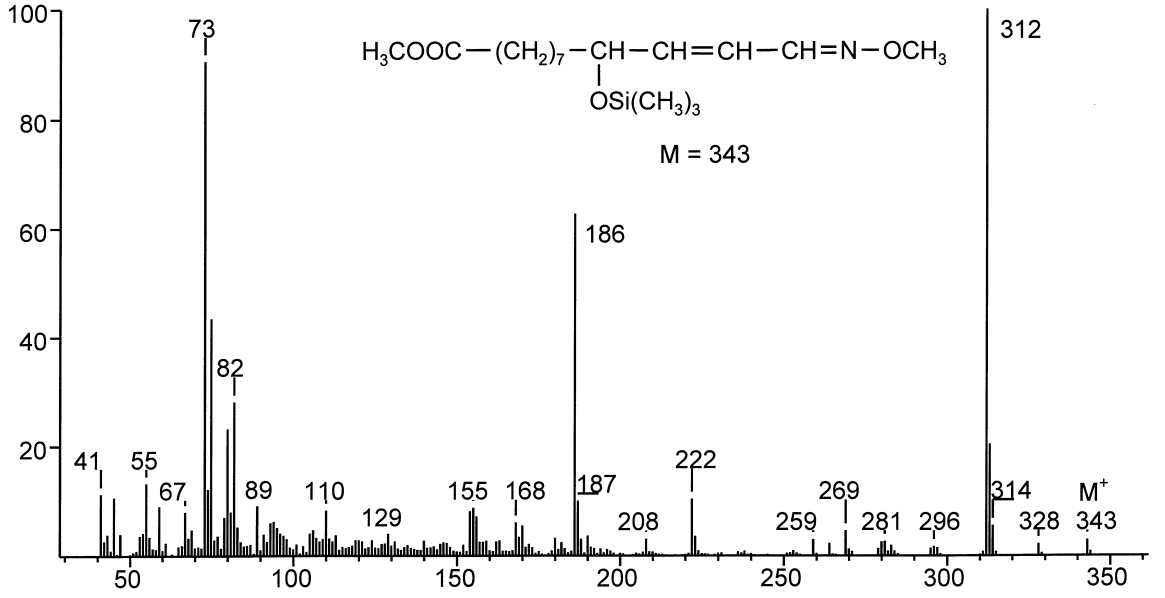


Fig. 93. Mass spectrum of the methoxime derivative of methyl-9-trimethylsilyloxy-12-oxo-10-dodecenoate* ($I = 2054$*).

(Fig. 93) is very simple: A main fragment is generated by loss of OCH_3 (m/z 312). This ion is degraded by expulsion of $(CH_3)SiOH$ to a N-substituted pyridinium ion (m/z 222). The latter ion in turn suffers loss of the side chain as $CH_2=CH-(CH_2)_4-COOCH_3$ to form the pyridinium ion (m/z 80).

The spectrum of pentafluorobenzylhydroxylamine derivative of methyl 9-trimethylsilyloxy-12-oxo-10-dodecenoate (Fig. 94) is characterized by three main peaks: α-cleavage occurs exclusively by loss of $(CH_2)_7-COOCH_3$ (m/z 352), in addition the fragments corresponding to loss of 181 u (m/z 328) and 197 u (m/z 312) are very prominent.

5.14. Final degradation products obtained by LPO of PUFAs

A final degradation product of PUFAs is malondialdehyde which occurs mainly as enol. This enol reacts with MSTFA to the adduct of the trimethylsilylated enol. Its mass spectrum shows an intense molecular ion peak at m/z 343. Even more pronounced are the fragments indicating the loss of a methyl group (m/z 328), a OTMS group (m/z 254), or CF_3 (m/z 274) and $N^{\cdot}(CH_3)-COCF_3$ (m/z 217). In addition, the typical ions for a MSTFA adduct of m/z 110,134 and 184 are recognized (Fig. 95).

In contrast, the enol of malondialdehyde generates readily by reaction with pentafluorobenzylhydroxylamine a bis-PFBO derivative. A prominent peak at m/z 250 in its mass spectrum (Fig. 96) may simulate the presence of a 2-alkenal – but in fact this ion is generated by loss of a molecule of pentafluorobenzylmethylether in the course of a rearrangement reaction. Best suited for identification of malondialdehyde seems the bis-methoxime derivative. Its mass spectrum shows an abundant molecular ion (Fig. 97).

A further degradation product of malondialdehyde is 3-oxopropanoic acid. Its mass spectrum (Fig. 98) is not very informative, since nearly only the ion of the pentafluorobenzylcation is generated.

A little more information is provided by the mass spectrum of the PFBO derivative of trimethylsilyl-3-oxopropanoate (Fig. 99). It shows besides the ion of mass 181 a M-15 fragment, this suffers further degradation by loss of CO_2 (m/z 296).

Other final degradation products of PUFAs are glyoxal and glyoxylic acid. Pentafluorobenzylhydroxylamine reacts with glyoxal twice, generating a bis-pentafluorobenzylhydroxylamine derivative. The mass spectrum of the latter shows only

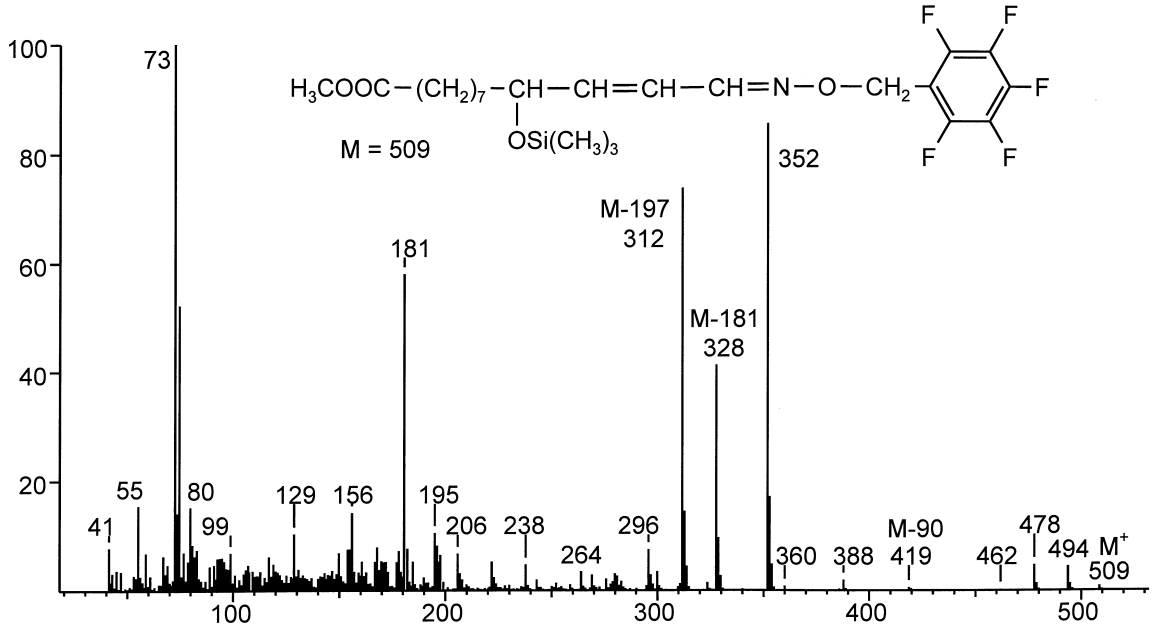


Fig. 94. Mass spectrum of the PFBO derivative of methyl-9-trimethylsilyloxy-12-oxo-10-dodecenoate*, see also Mlakar [108] for COO-TMS derivative ($I_1 = 2556$*; $I_2 = 2565$*).

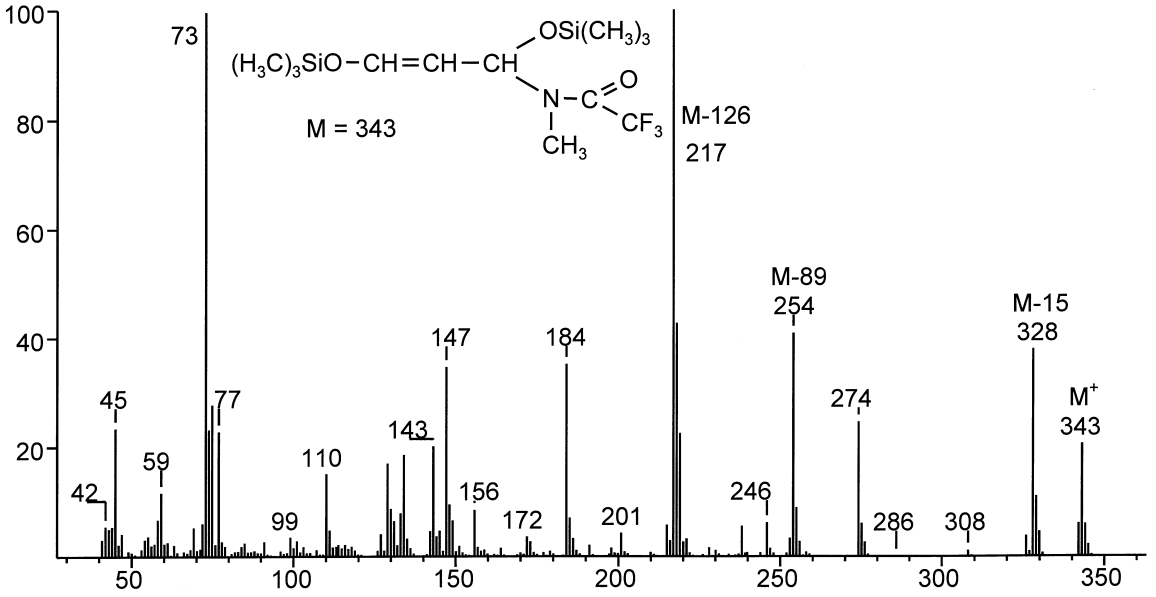


Fig. 95. Mass spectrum of the MSTFA adduct derived from the trimethylsilylated enol of malondialdehyde ($I = 1375$), see Mlakar [108].

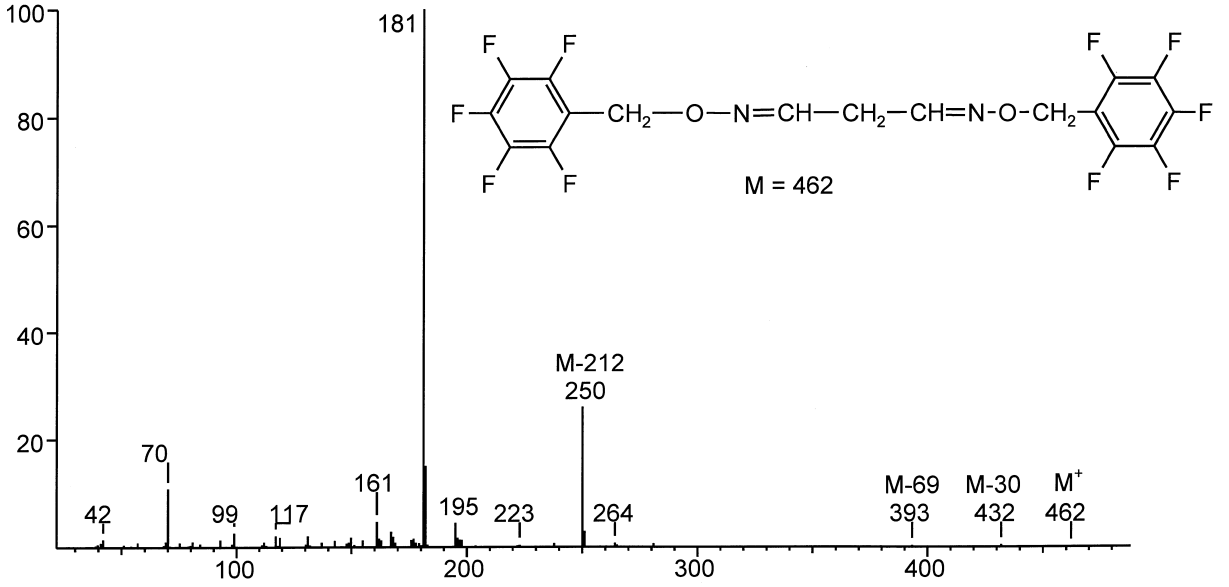


Fig. 96. Mass spectrum of the *bis*-PFBO derivative obtained from malondialdehyde ($I=1988$).

two peaks: That of the pentafluorobenzyl ion of mass 181 and a weak molecular ion (m/z 448, Fig. 100).

Reaction of glyoxal with MSTFA generates several products: The bis-MSTFA adduct is obtained but only in moderate yield. Its mass spectrum (Fig. 101) is characterized by the typical MSTFA adduct peaks (m/z 110, 134, 184 and 228). Rather intense is a fragment generated by loss of a methyl radical

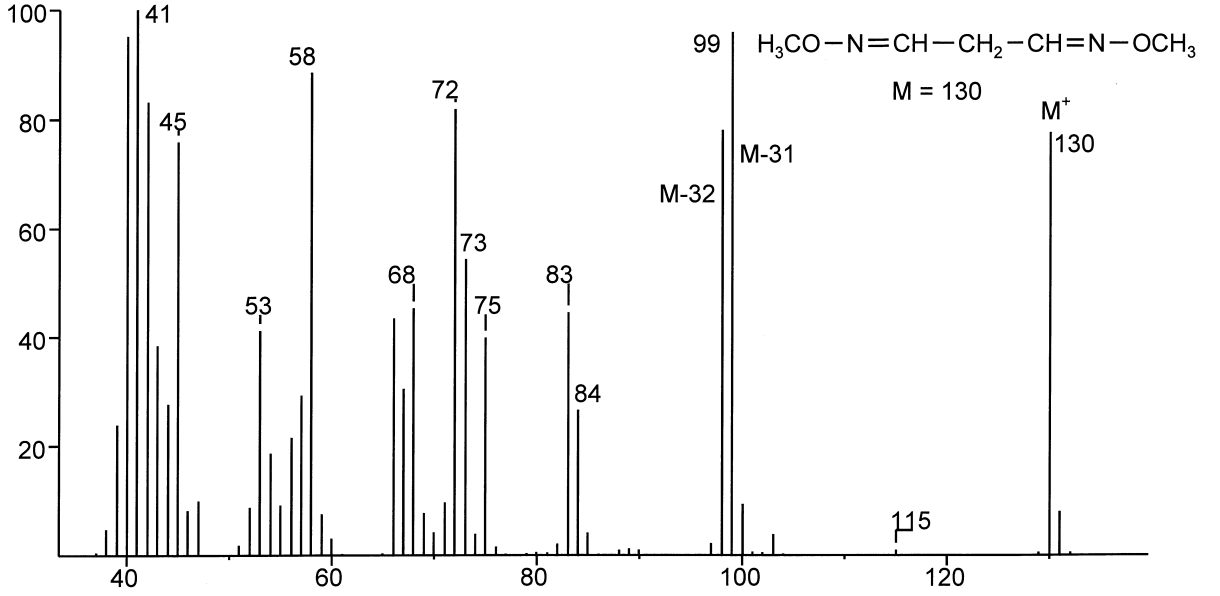


Fig. 97. Mass spectrum of the *bis*-methoxime derivative obtained from malondialdehyde ($I=964$).

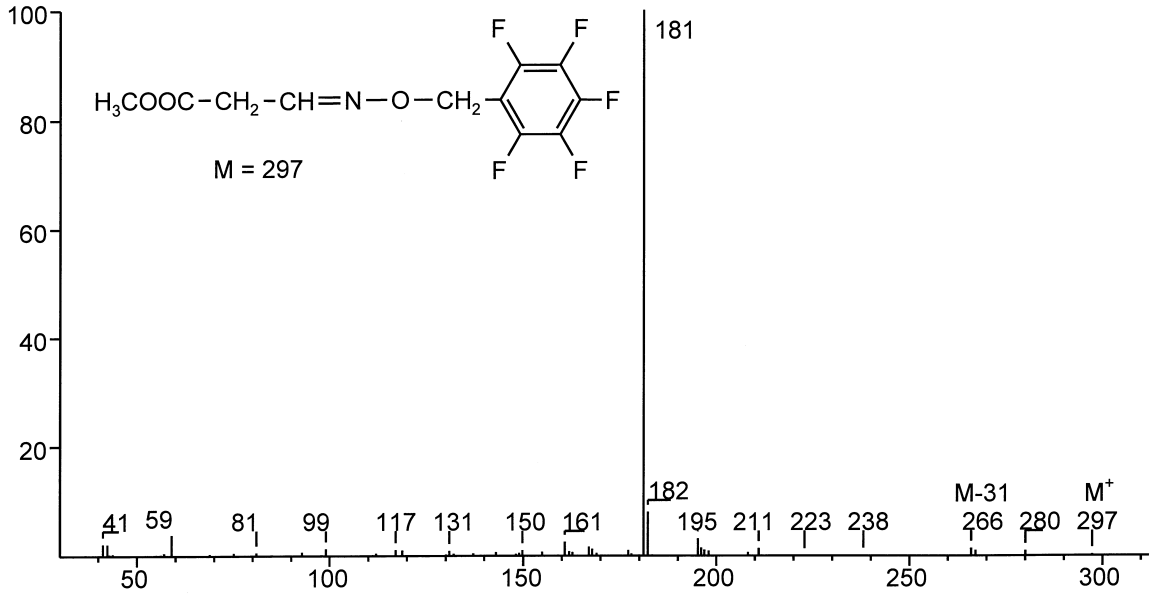


Fig. 98. Mass spectrum of the PFBO derivative of methyl-3-oxopropanoate ($I = 1353$).

followed by expulsion of $CH_3N{=}C(OH)\text{-}CF_3$ (m/z 314, M-142).

Another main product is obtained by addition of MSTFA to one aldehydic group and formation of a trimethylsilylacetale with the other. Also a 1,1,2,2-tetratrimethylsilyloxyethane is generated rendering trimethylsilylation in this case a problematic reaction.

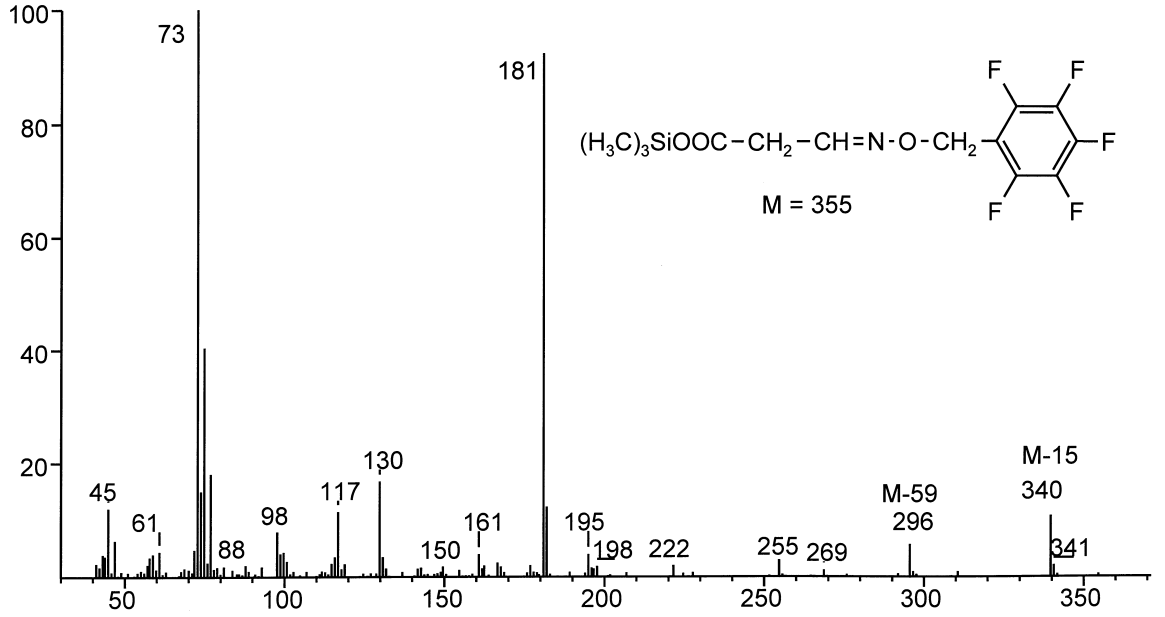


Fig. 99. Mass spectrum of the PFBO derivative of trimethylsilyl-3-oxopropanoate ($I = 1479$).

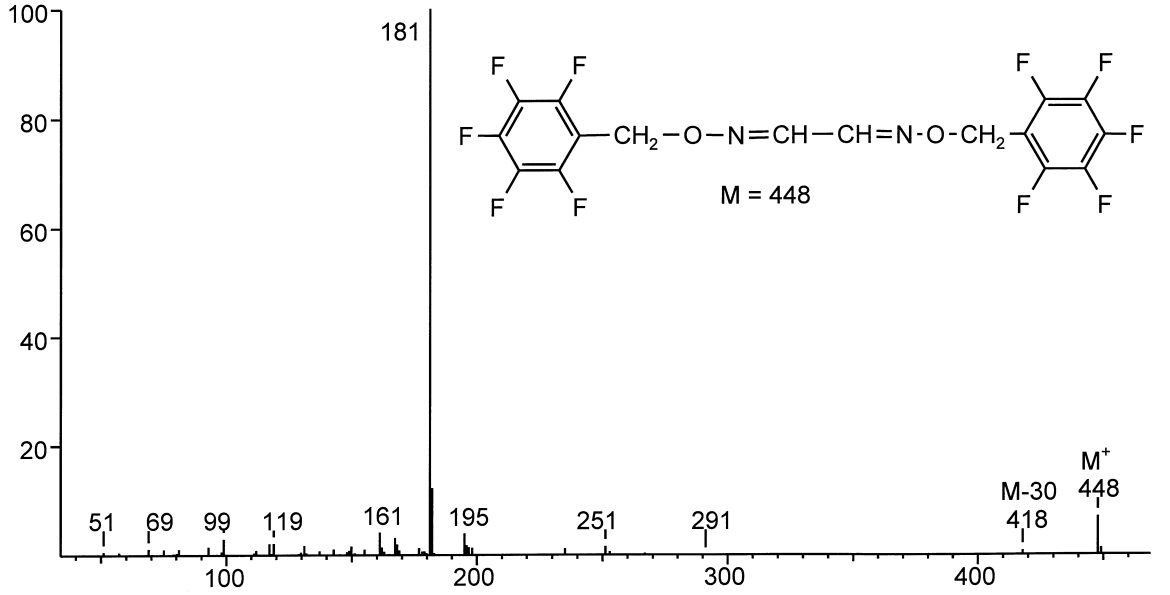


Fig. 100. Mass spectrum of the *bis*-pentafluorobenzylhydroxylamine derivative of glyoxal ($I_1 = 1916$; $I = 1925$), see also Loidl-Stahlhofen [87] and Mlakar [108].

Methylglyoxalate generates by treatment with MSTFA two derivatives, the usual MSTFA adduct, but also a trimethylsilyl acetale. The mass spectrum of the first (Fig. 102) shows a M−15 ion and the fragments of mass 110, 134, 184, 228 and M−126, typical for MSTFA adducts, the latter (Fig. 103) is characterized by a M−15 ion (m/z 235) and a M−59 ion of mass 191. The M−15 ion is degraded

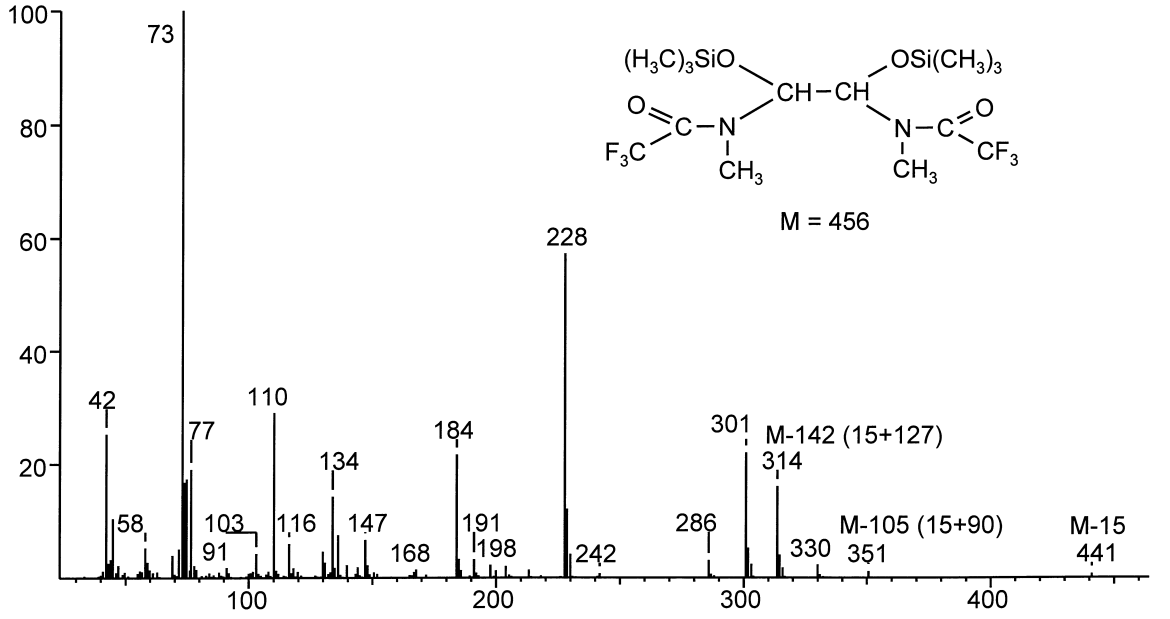


Fig. 101. Mass spectrum of the *bis*-MSTFA adduct derived from glyoxal ($I_1 = 1415$; $I_2 = 1464$).

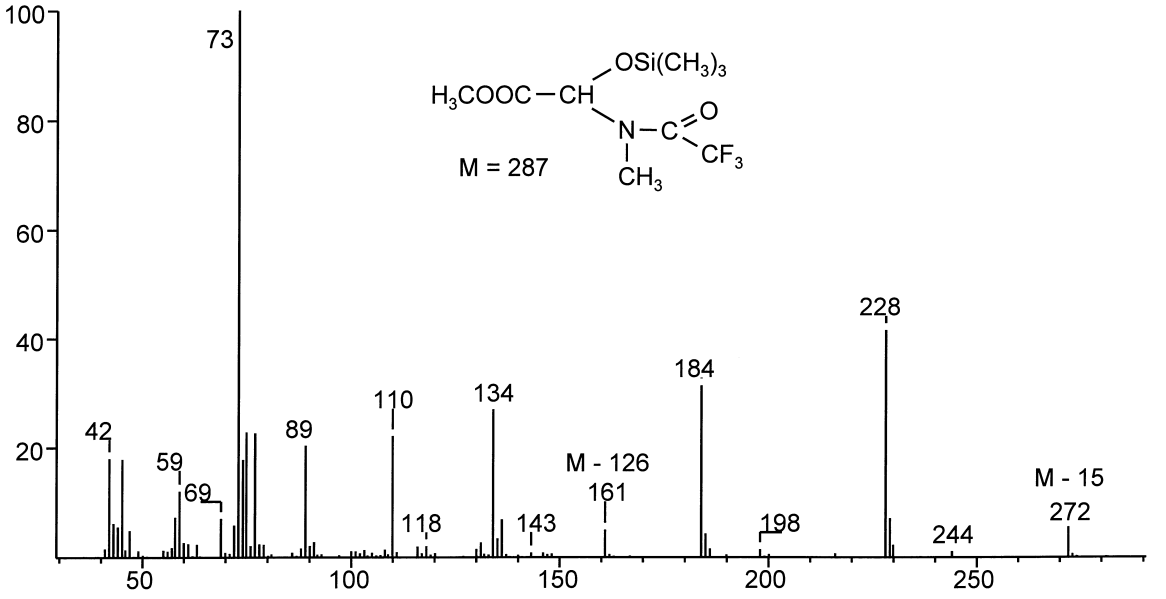


Fig. 102. Mass spectrum of the MSTFA adduct derived from methylglyoxylate ($I=1143$).

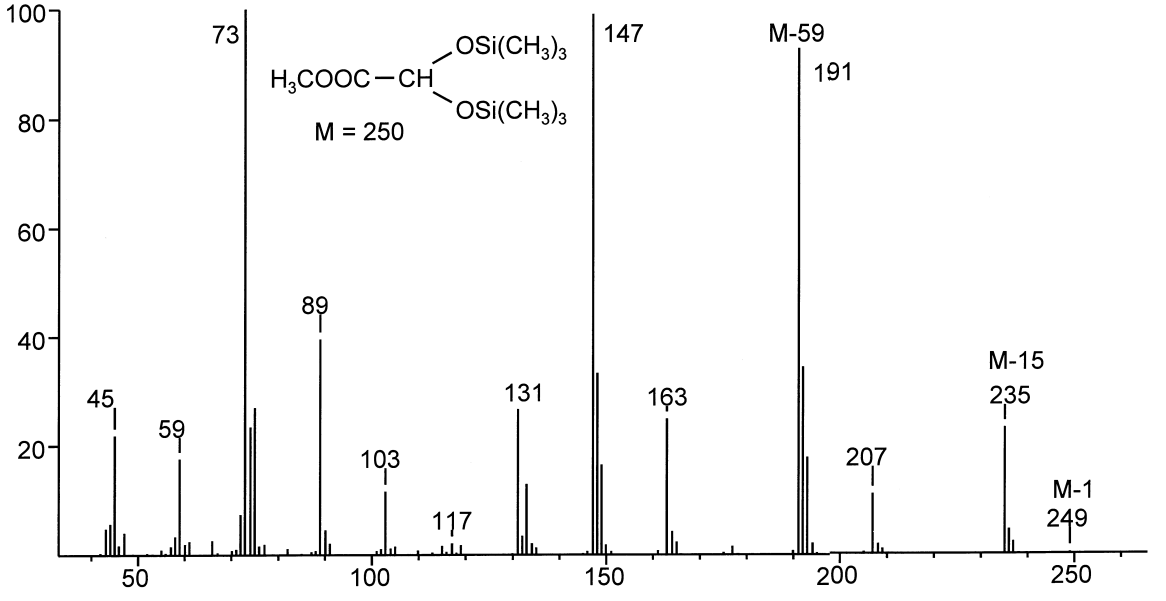


Fig. 103. Mass spectrum of 2,2-*bis*-trimethylsilyloxy-methylglyoxalate ($I=1169$).

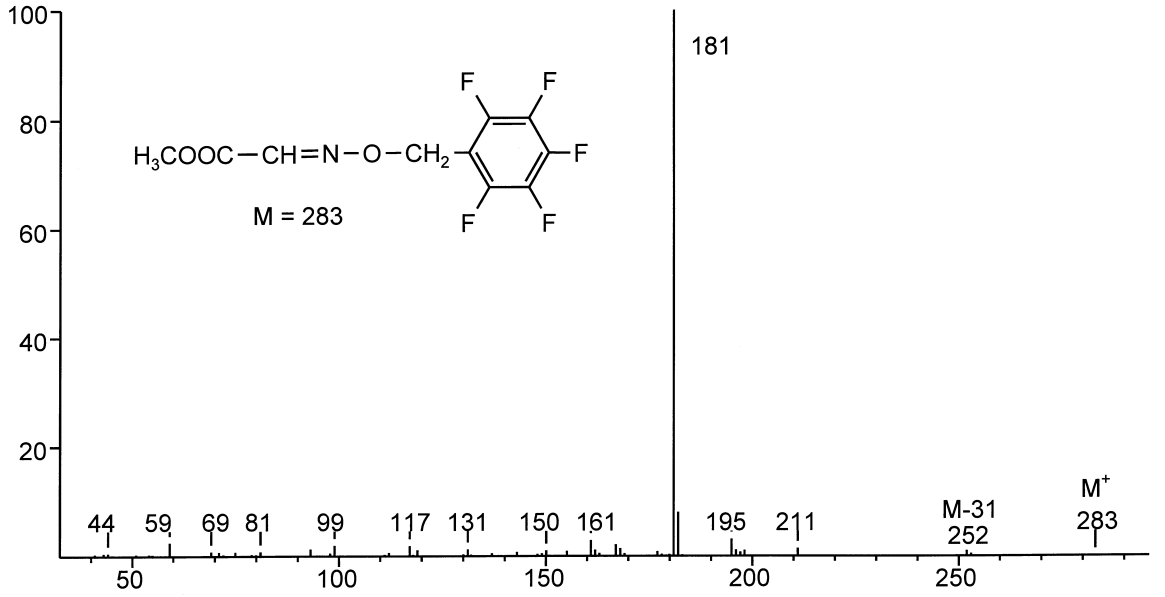


Fig. 104. Mass spectrum of the PFBO derivative of methylglyoxylate ($I=1353$).

by loss of a particle of 72 u $(CH_3)_2Si=CH_2$ (m/z 163). In addition, an ion of mass 147 indicates presence of two trimethylsilyl residues.

The MS of the PFBO derivative of methylglyoxylate is not very informative: It shows besides the dominating ion of mass 181 only tiny peaks (Fig. 104), not allowing any structural assignments. In contrast the spectrum of the TMS ester does show a weak molecular ion and a M−15 ion, rendering compound identification easier (Fig. 105).

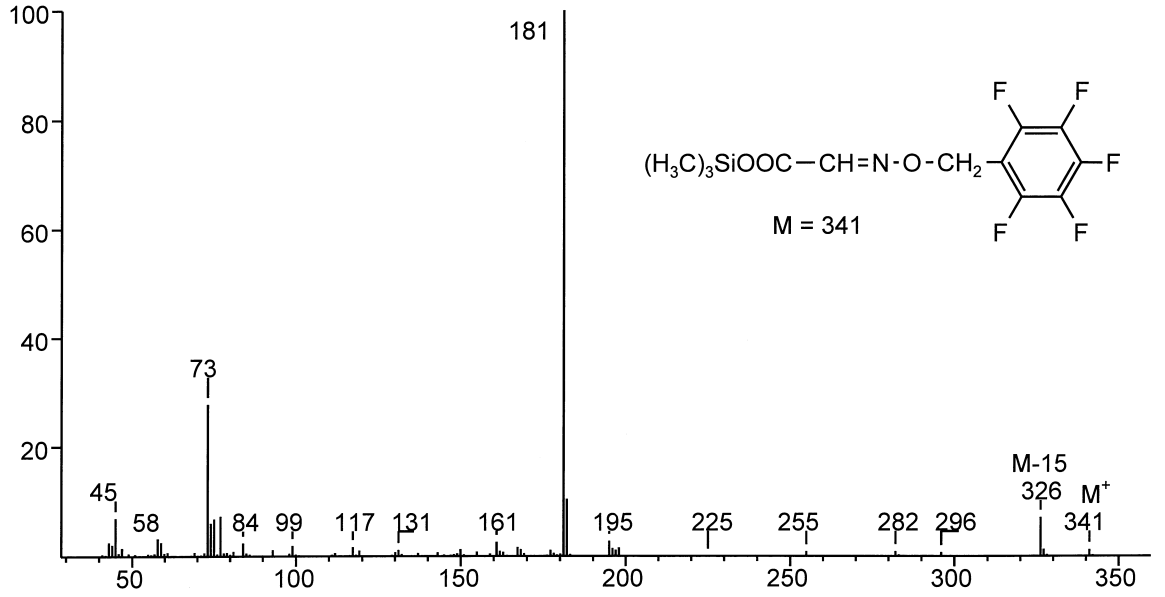


Fig. 105. Mass spectrum of the PFBO derivative of trimethylsilyl-glyoxylate ($I=1476$).

6. Conclusion

Aldehydes of low mass are easily lost in the course of sample processing. In order to restrict these losses their early transformation into less volatile derivatives is required. Unfortunately most derivatives of simple compounds, e.g. saturated aldehydes, give mass spectra lacking molecular ions. The spectra are also poor in fragment ions with structural specifity. In these cases preparation of 4-nitrophenylhydrazones is recommended, although the reaction between aldehydes and the reagent is not as fast as that with pentafluorobenzylhydroxylamine and ions in the mass spectra are registered with much less sensitivity (the high electron density in the aromatic ring of pentafluorobenzyl derivatives facilitates ionization).

Since functional groups induce specific degradation reactions the spectra become more instructive the more functional groups are present. Thus in contrast to mass spectra of PFBO derivatives of straight chain aldehydes those of unsaturated aldehydes and those with additional functional groups usually do not only show molecular ions, but key ions as well. In most cases preparation of PFBO derivatives is the best way to obtain information on the structure of an aldehydic compound. Pentafluorobenzylhydroxylamine reacts fast with aldehydic compounds, the derivatives are detected with high sensitivity. Investigation of aldehydic acids and hydroxyaldehydes requires a preceding protection of the polar groups by esterfication and/or trimethylsilylation.

The fastest way to prepare a suitable derivative is the reaction with MSTFA (or another trimethylsilylating reagent). This allows protection of OH and COOH functions in one step. A disadvantage of this method is the tendency of trimethylsilylesters to undergo hydrolysis, requiring exclusion of even traces of water. In the course of this reaction not only OH and COOH groups are protected, but also the CHO group adds MSTFA to form an adduct which usually provides also informative mass spectra. These give sometimes additional structural information. A disadvantage of the MSTFA adduct formation is the slow reaction, usually transformation to adducts is not quantitative. Thus in most cases a two or three step reaction is preferable, first the carbonyl groups are derived, followed by conversion of acidic groups into their methyl esters by short treatment with diazomethane and finally by reacting OH groups to trimethylsilyl derivatives. The choice of the reaction conditions is also important: The lower the carbonyl activity, the more enforced reactions condition (heat, increased reaction time) is required to obtain reasonable yields of the aldehydic derivatives. In addition not all derivatizing reagents react exclusively with the aldehydic group: Thus for instance methyl esters are able to react with hydrazine and its derivatives by formation of hydrazides. Other side reactions are observed with MSTFA which generates bis-trimethylsilyacetals or trimethylsilylenolethers.

For most purposes generation of pentafluorobenzyloximes seems the best choice. Nevertheless the heavy pentafluorobenzyl group may increase the molecular mass and thus reduce the volatility too much. As a consequence dialdehydes or ketoaldehydes, or substituted aldehydic compounds, are better transformed to methoximes which possess higher volatility compared to corresponding PFBO derivatives and give usually also informative mass spectra.

Biological material contains a very different spectrum of aldehydic LPO products, mostly only in traces. Due to above outlined properties of functionalized aldehydes their detection requires usually division of the sample: One sample is used to generate dimethylhydrazones or 4-nitrophenylhydrazones to obtain from straight chain aldehydes compounds which give characteristic mass spectra, one sample is derivatized with pentafluorobenzylhydroxylamine to identify most of the aldehydic compounds and a third one is transformed to methoximes to trap also aldehydes of low volatility and enable GC separation in reasonable time. If the above outlined facts are considered separation and identifications of LPO aldehydes become easy by GC–MS.

Acknowledgements

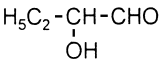
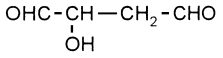
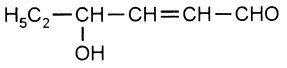
We thank Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for financial support.

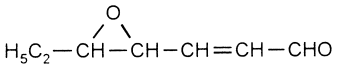
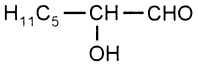
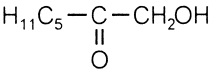
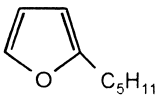
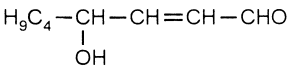
Appendix 1

Mass spectra of simple aldehydic compounds are recorded in spectra collections [43] or [44]. They are also stored in computer search programs of different MS manufactures. Mass spectra of derivatives of natural occurring aldehydic compounds are rarely available. Information about aldehydic compounds obtained by lipid peroxidation of PUFAs are listed in the following table according to increasing molecular mass of the unsubstituted aldehyde. The table informs about molecular structure and provides references to spectral data. Molecular masses of derivatives are included in parenthesis. It must be emphasized that we had not been able to check the validity of published data, some of the reported compounds have not been detected in samples of biological origin in our laboratory. Suspected artificial generation of compounds is indicated in form of a comment. Abbreviations: CI, chemical ionization; DNPH, 2,4-dinitrophenylhydrazone; EI, electron impact; MS, mass spectrum; PFBO, pentafluorobenzyloxime; TMS, trimethylsilyl; MSTFA, N-methyl-N-trimethylsilyltrifluoroacetamide.

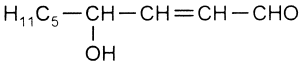
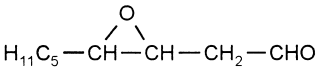
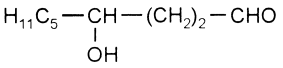
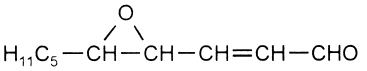
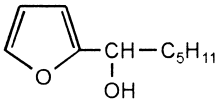
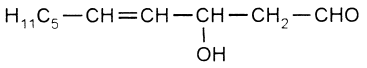
Mass	Formula	Name	Ref.	Comment
44	H_3C-CHO	**Acetaldehyde**:		
		EI-MS	[103]	
		CI-MS of DNPH derivative (224)	[59]	
		EI-MS of DNPH derivative (224)	[113]	
56	H_2C=CH-CHO	**Acrolein**:		
		CI-MS of DNPH derivative (236)	[59]	
58	H_3C-CH_2-CHO	**Propanal**:		
		EI-MS	[103]	
		EI-MS of DNPH derivative (238)	[113]	
		CI-MS of DNPH derivative (238)	[59]	
		EI-MS of MSTFA adduct (257)	[108]	
58	OHC-CHO	**Glyoxal**:		
		EI-MS of PFBO derivative (448)	[87]	
			[108]	
70	H_3C-CH=CH-CHO	**2-Butenal**:		
		EI-MS of benzyloxime derivative (175)		Sometimes artifact,
		LC-MS of DNPH derivative (250)	[126]	generated from
		CI-MS of DNPH derivative (250)	[59]	2-hydroxybutanal
72	H_3C-$(CH_2)_2$-CHO	**Butanal**:		
		EI-MS	[103]	
		EI-MS of oxime derivative (87)	[136]	
		EI-MS of semicarbazone (129)	[115]	
		EI-MS of dimethylhydrazone (114)	[114]	
		CI-MS of DNPH derivative (252)	[59]	
		EI-MS of DNPH derivative (252)	[113]	
72	OHC-CH_2-CHO	**Malondialdehyde**:		
		EI-MS of TMS enolether-		
		MSTFA adduct derivative (343)	[108]	
		negative ion CI-MS of PBFO		
		derivative (462)	[70]	
84	H_5C_2-CH=CH-CHO	**2-Pentenal**:		
		LC-MS of DNPH derivative (264)	[126]	
		CI-MS of DNPH derivative (264)	[59]	
		EI-MS of benzyloxime derivative (189)	[64]	
			[131]	
		EI-MS of MSTFA adduct (283)	[108]	

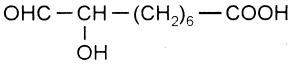
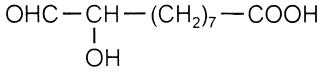
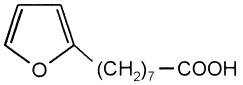
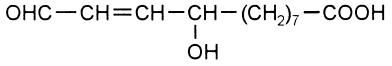
(*Continued overleaf*)

Mass	Formula	Name	Ref.	Comment
86	H_3C-$(CH_2)_3$-CHO	**Pentanal**:		
		EI-MS	[103]	
		EI-MS of oxime derivative (101)	[136]	
		EI-MS of semicarbazone (143)	[115]	
		EI-MS of dimethylhydrazone (128)	[114]	
		EI-MS of DNPH derivative (266)	[113]	
		EI-MS of o− and p-NPH (221)	[111]	
		EI-MS of DNPH derivative (266)	[111]	
		CI-MS of DNPH derivative (266)	[59]	
88		**2-Hydroxybutanal**:		
		EI-MS of TMS/PFBO derivative (355)	[108]	
		EI-MS of TMS/MSTFA adduct (359)	[108]	
98	H_7C_3-CH=CH-CHO	**2-Hexenal**:		
		EI-MS of benzyloxime derivative(203)	[64]	
		CI-MS of DNPH derivative (278)	[59]	
98	H_7C_3-CH=CH-CHO	**3-Hexenal**:		
		EI-MS of benzyloxime derivative (203)	[65]	
100	$H_{11}C_5$-CHO	**Hexanal:**		
		EI-MS	[103]	
			[138]	
			[137]	
			[122]	
		EI-MS of DNPH derivative (280)	[113]	
		CI-MS of DNPH derivative (280)	[59]	
		negative ion CI-MS of PBFO derivative (295)	[70]	
102	HO-$(CH_2)_4$-CHO	**5-Hydroxypentanal**:		
		EI-MS of DNPH derivative (282)	[59]	
		CI-MS of TMS-DNPH derivative (354)	[59]	
102		**2-Hydroxysuccinaldehyde**:		
		EI-MS of TMS-bis-PFBO derivative (564)	[124]	
110	OHC-CH=CH-CH=CH-CHO	**Mucondialdehyde**:		
		EI-MS	[139]	
110	H_5C_2-CH=CH-CH=CH-CHO	**2,4-Heptadienal**:		
		MS of PFBO derivative (305)	[108]	
		MS of MSTFA adduct (309)	[108]	
112	H_9C_4-CH=CH-CHO	**2-Heptenal**:		
		CI-MS of DNPH derivative (292)	[59]	
		LC-MS of DNPH derivative (292)	[126]	Often artifact, derived by dehydration of 2-hydroxyheptanal
112	H_5C_2-CH=CH-$(CH_2)_2$-CHO	**4-Heptenal**:		
		CI-MS of DNPH derivative (292)		
114		**4-Hydroxy-2-hexenal**:		
		EI-MS	[25]	
		MS of TMS-benzyloxime derivative (291)	[65]	
		MS of TMS-PFBO derivative (381)	[108]	
114	$H_{13}C_6$-CHO	**Heptanal**:		
		EI-MS	[103]	
		EI-MS of DNPH derivative (294)	[113]	
		CI-MS of DNPH derivative (294)	[59]	

Mass	Formula	Name	Ref.	Comment
124	H_5C_2-CH=CH-CH_2-CH=CH-CHO	**2,5-Octadienal**:		
		EI-MS	[138]	
126		**4,5-Epoxy-(*E*)-2-heptenal**:		
		EI-MS	[140]	
		EI-MS of methoxime derivative (155)	[140]	
126	$H_{11}C_5$-CH=CH-CHO	**2-Octenal**:		
		EI-MS	[138]	
		EI-MS of 2,3-dideuterated derivative (128)	[31]	
		CI-MS of DNPH derivative (306)	[59]	
128	H_3C-$(CH_2)_6$-CHO	**Octanal**:		
		EI-MS of DNPH derivative (308)	[113]	
130		**2-Hydroxyheptanal**:		
		EI-MS of t-BDMS-PFBO derivative (439)	[81]	
		EI-MS of TMS-PFBO derivative (397)	[87]	
		EI-MS TMS-MSTFA adduct (401)	[87]	
130		**1-Hydroxy-2-heptanon**:		
		EI-MS	[25]	Artifact, due to thermal rearrangement of 2-hydroxyheptanal
138	H_9C_4-CH=CH-CH=CH-CHO	**2,4-Nonadienal**:		
		CI-MS of DNPH derivative (318)	[59]	Probably artifact, generated from 4-hydroxy-2-nonenal
138	H_5C_2-CH=CH-$(CH_2)_2$-CH=CH-CHO	**2,6-Nonadienal**:		
		CI-MS of DNPH derivative (318)	[59]	
138		**2-Pentylfuran**:		
		EI-MS	[122]	Probably artifact generated from 4-hydroxy-2-nonenal
140	$H_{13}C_6$-CH=CH-CHO	**2-Nonenal**:		
		EI-MS	[137,141]	
		LC-MS of DNPH derivative (320)	[126]	
142	$H_{17}C_8$-CHO	**Nonanal**:		
		EI-MS	[103]	
		EI-MS of DNPH derivative (322)	[113]	
142		**4-Hydroxy-2-octenal**:		
		EI-MS	[25]	
144	$H_{13}C_6$–CH(OH)–CHO	**2-Hydroxyoctanal**:		
		EI-MS of TMS-PFBO derivative (411)	[108]	
150	H_5C_2-CH=CH-CH_2-$(CH=CH)_2$-CHO	**2,4,7-decatrienal**:		
		EI-MS of benzyloxime derivative (255)	[142]	
152	$H_{11}C_5$-$(CH=CH)_2$-CHO	**2,4-Decadienal**:		
		EI-MS	[122]	
		CI-MS of DNPH derivative (332)	[59]	

(*Continued overleaf*)

Mass	Formula	Name	Ref.	Comment
156	$H_{19}C_9$-CHO	**Decanal**:		
		EI-MS	[103]	
		CI-MS of DNPH derivative (336)	[59]	
156		**4-Hydroxy-2-nonenal**:		
		EI-MS	[25]	
		EI-MS of TMS O-benzyloxime	[65]	
		derivative (333)	[143]	
		negative ion CI-MS of TMS/PFBO	[144]	
		derivative (423)	[37]	
			[70]	
			[68]	
		EI-MS of N-methylpyrazoline (184)	[88]	
156		**3,4-Epoxynonanal**:		
		EI-MS	[143]	Mass spectrum not in agreement with deduced structure
158		**4-Hydroxynonanal**:		
		EI-MS of TMS/PFBO derivate (425)	[97]	
158	OHC-$(CH_2)_6$-COOH	**8-Oxooctanoic acid**:		
		EI-MS of methylester (172)	[32]	
			[145]	
			[133]	
168		**4,5-Epoxy-2-decenal**:		
		CI-MS	[121]	
		EI-MS	[122]	EI-MS data not in agreement with other references
		EI-MS	[123]	
			[133]	
168		**1-(2-Furyl)-hexan-1-ol**:		
		EI-MS	[25]	Assumed cyclization product of 4,5-dihydroxy-2-decanal.
170		**3-Hydroxy-4-decenal**:		
		EI-MS	[121]	
172	OHC-$(CH_2)_7$-COOH	**9-Oxononanoic acid**:		
		EI-MS of methylester (186)	[145]	
			[32]	
		EI-MS of MSTFA adduct derived from TMS (443)	[108]	
		negative ion CI-MS of PBFO derivative of methylester (381)	[144]	

Mass	Formula	Name	Ref.	Comment
184	OHC-CH=CH-$(CH_2)_6$-COOH	**10-Oxo-8-decenoic acid**:		
		EI-MS of methylester (198)	[138]	
			[32]	
		CI-MS	[146]	
184	$H_{23}C_{11}$-CHO	**Dodecanal**:		
		CI-MS of DNPH derivative (364)	[59]	
186	OHC-$(CH_2)_8$-COOH	**10-Oxodecanoic acid**:		
		EI-MS of methylester (200)	[138]	
188		**9-Oxo-8-hydroxynonanoic acid**:		
		EI-MS of t-BDMS-PFBO methylester (511)	[81]	
198	OHC-CH=CH-$(CH_2)_7$-COOH	**11-Oxo-9-undecenoic acid**:		
		EI-MS of methylester (212)	[32]	
202		**9-Hydroxy-10-oxodecanoic acid**:		
		EI-MS of PFBO derivative of TMS ester (541)	[108]	
210		**8-(2-Furyl)-octanoic acid**:		
		EI-MS of methylester (224)	[133]	Probably cyclization
			[147]	product derived from 12-oxo-9-hydroxy-10-dodecenoic acid
212	OHC-CH=CH-$(CH_2)_8$-COOH	**12-Oxo-10-dodecenoic acid**:		
		EI-MS of methylester (226)	[137]	
			[148]	
			[32]	
			[149]	
		EI-MS of methylester-methoxime		
		derivative (241)	[149]	
212	OHC-CH_2-CH=CH-$(CH_2)_7$-COOH	**12-Oxo-9-dodecenoic acid**:		
		EI-MS of methylester (255)	[32]	
			[148]	
214	OHC-$(CH_2)_{10}$-COOH	**12-Oxododecanoic acid**:		
		EI-MS of TMS-enolether-methylester (300)	[150]	
			[131]	
224	OHC-CH=CH-CH=CH-$(CH_2)_7$-COOH	**13-Oxo-9,11-tridecadienoic acid**:		
		EI-MS of methylester (238)	[133]	
			[139]	
			[147]	
			[132]	
		EI-MS of DNPH derivative	[151]	
		of methylester (418)		
228		**9-Hydroxy-12-oxo-10-dodecenoic acid**:		
		EI-MS of TMS/PFBO derivative		
		of TMS ester (567)	[108]	

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