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Gas chromatographic–electron impact mass spectrometric screening procedure for unknown hydroxyaldehydic lipid peroxidation products after pentafluorobenzyloxime derivatization

Angelika Loidl-Stahlhofen, Werner Kern, Gerhard Spiteller*

Lehrstuhl Organische Chemie I, Universität Bayreuth, NW I, Universitätsstrasse 30, 95440 Bayreuth, Germany

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Abstract

Aldehydic lipid peroxidation products can be detected after transformation to pentafluorobenzyloxime derivatives by GC–MS screening using characteristic ion traces. Thus the rather unstable unsaturated hydroxyaldehyde, 6-hydroxy-2,4-undecadienal, was identified as autoxidation product of linoleic acid. Its structure was unambiguously confirmed by comparison with an authentic sample. After Fe^{2+}-ascorbate induced lipid peroxidation of oleic acid several 4-hydroxy-2-alkenals and 4-hydroxyalkanals were detected. These represent previously unknown secondary oxidation products of lipid peroxidation of oleic acid. Nevertheless oleic acid proved about 1000 times more stable against peroxidation than linoleic or higher unsaturated acids.

1. Introduction

In recent years more and more evidence was accumulated that inflammation processes [1,2], aging [3,4], ischemia [5] and atherosclerosis [6,7] are connected with oxidative stress and enhanced lipid peroxidation (LPO) [8–11], which is also observed during tissue injury [12]. Hydroperoxides of unsaturated fatty acids (LOOHs) easily undergo secondary reactions [11,13–15]. β-Cleavage results in formation of aldehydes [11,14–18].

The extent of lipid peroxidation is usually measured spectrophotometrically by determination of an 1:2 adduct of malondialdehyde (MDA) —a decomposition product of LOOHs— with thiobarbituric acid (TBA) [19]. However, this reaction is neither specific nor quantitative [20]; it must be emphasized that especially hydroperoxides produced from linoleic acid —representing a high percentage of in vivo occurring LPO products [21]— escape detection.

Better quantitative results may be obtained applying aldehyde-specific derivatization methods followed by separation of the corresponding derivatives by chromatography. Thus Esterbauer and Schauenstein [22] detected the important cytotoxic LPO product 4-hydroxy-2-nonenal (4HNE) by preparation of 2,4-dinitrophenylhydrazone derivatives. This method later allowed the identification of some additional saturated and unsaturated aldehydes [17,23,24] in

* Corresponding author.

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LPO mixtures and also of osazones which were assumed to be derivatives of α,β-diketones or acyloins [17,25,26]. Later we have been able to show that these products are derived from α-hydroxyaldehydes [27–29].

If separation of 2,4-dinitrophenylhydrazones is achieved by thin layer chromatography (TLC) [17,24,25], rather large amounts of sample are required to isolate single compounds, if only HPLC is applied separation is often incomplete.

Therefore the combination of gas chromatography (separation) and mass spectrometry (identification) for detection of samples occurring only in trace amounts is indicated. Unfortunately 2,4-dinitrophenylhydrazones of aldehydes with molecular masses higher than 200 are of low volatility, rendering their separation by GC difficult. In addition, interpretation of their mass spectra is complicated by rearrangement reactions [30,31]. Therefore preparation of 2,4-dinitrophenylhydrazones is not advisable for application of GC–MS.

Dimethylacetals of aldehydes are easily available, they are of high volatility, but their mass spectra give only scarce information. They often lack molecular ions; the base peak is caused by a predominant α-cleavage reaction at the acetal group resulting in a fragment $m/z = 75$ [32].

In contrast, mass spectra of dithioethyleneglycolacetals usually show molecular ions and other structure-specific fragments [33,34], but their preparation conditions (BF_3 is needed) are rather drastic and often lead to unexpected side reactions [35]. In addition, the procedure is time-consuming.

Pentafluorobenzylhydroxylamine is widely used in GC–MS for derivatization of carbonyl compounds [36,37]: the presence of five fluor atoms makes pentafluorobenzyloxime (PFBO) derivatives especially useful for GC detection by electron capture detectors. They are also perfectly suited for detection by negative ion mass spectrometry combined with single ion monitoring [36]. Unfortunately using this method mainly $(M-1)$ ions or other key ions are indicated, rendering the identification of unknown compounds difficult or even impossible.

Considering all these facts we worked out an GC–EI-MS screening procedure for aldehydic lipid peroxidation products after pentafluorobenzyloxime derivatization. Applying this method we were able to detect a great number of previously unknown unsaturated oxo acids and several α-hydroxyaldehydic compounds after lipid peroxidation of linoleic and oleic acid [27,28,38] by registration of characteristic key ions (Table 1).

In this paper we report the detection of previously unknown aldehydes derived by lipid peroxidation of linoleic and oleic acid. Their occurrence was predicted according to a general mechanism recently presented [28].

2. Experimental

2.1. Materials

N-Methyltrimethylsilytrifluoracetamide (MSTFA) was obtained from Macherey and Nagel (Düren, Germany). Silica gel 60 PF_{254} was obtained from Merck (Darmstadt, Germany), home-made TLC plates were used. All other chemicals were purchased from Fluka (Neu Ulm, Germany). The fatty acids and 6-hydroxy-2,4-undecadienal were stored at −18°C. 1-Hydroxy-2-dodecanone [36] and the PFBO derivative of 2-hydroxyundecanal [31,37] were prepared as described previously.

2.2. Synthesis of 6-hydroxy-2,4-undecadienal *3a*

Synthesis of 6-hydroxy-2,4-undecadienal **3a** was achieved via appropriate transformation of methods for synthesis of 4-hydroxy-2-alkenals **2** [38–42]. Reaction products were characterized by TLC and GC, structure confirmation was performed by GC–MS and ^{1}H-NMR.

(a) Furfuraltosylhydrazone **5** was prepared according to Foster and Agosta [41]. Furfural **4** (13.06 g, 136 mmol) and tosylhydrazine (25.3 g, 136 mmol) were suspended in 100 ml absolute methanol and stirred at room temperature until a clear solution was obtained. By cooling (argon atmosphere) to −4°C (refrigerator) **5** precipitated. It was isolated by filtration, dried in

Table 1
EI-MS key fragments of different classes of aldehydes as O-PFB oxime TMS-ether derivatives

class of compounds	key fragments (m/z)
alkanals R–CH=PFBO	181, 239, M^+
2-alkenals R–CH=CH–CH=PFBO	181, 250, M^+
2,4-alkadienals R–CH=CH–CH=CH–CH=PFBO	181, 276, M^+
n-oxo-acids (me) $H_3CO{>}C(=O)-(CH_2)_x-CH=PFBO$	181, 239, M–31, (1 db: 250, 2 db: 276)
2-hydroxyalkanals R – CH(OTMS)–CH=PFBO	181, 326, M–15, M–181, M–197
4-hydroxy-2-alkenals R – CH(OTMS)–CH=CH–CH=PFBO	181, 352, M–15, M–181, M–197
(n–1)-hydroxy-n-oxo acids (me) $H_3CO{>}C(=O)-(CH_2)_x-CH(OTMS)-CH=PFBO$	181, 326, M–15, M–181, M–197, 74, M–31, M–32–181
(n–3)-hydoxy-n-oxo-(n–2)-en acids (me) $H_3CO{>}C(=O)-(CH_2)_x-CH(OTMS)-CH=CH-CH=PFBO$	181, 352, M–15, M–181, M–197, 74, M–31, M–32–181

vacuum and crudely purified by TLC [cyclohexane (CH), ethylacetate (EA)]. The raw material was used without further purification for preparation of pent-*trans*-2-en-4-ynal **8**.

Yield: 34.3 g (= 95.5%); TLC (CH–EA, 1:1): $R_F = 0.59$; GC (OV 101): $R_i = 2182/2214$ (*anti*/*syn*); MS (70 eV): *m*/*z* (%) = 81(100), 80(84), 52(38), 108(28), 91(24), 264(23).

(b) Pent-*trans*-2-en-4-ynal **8** was prepared following the procedures of Esterbauer and Weber [42] and Shapiro [43]. An aliquot of 2 g (83.3 mmol) sodium hydride (100%) was added to 23.8 g (90.2 mmol) furfuraltosylhydrazone **5**, dissolved in 170 ml dry CH_2Cl_2. The solution was stirred under argon, until hydrogen formation stopped (ca. 30 min). By using a stream of argon the solvent was removed nearly completely. A white precipitate of the sodium salt of **5** 24.8 g (26.7 mmol) remained after vacuum drying. The salt was carefully and slowly heated on a sand bath applying vacuum [0.1 Torr (ca. 13 Pa)]. At 105°C spontaneous decomposition occurred. Compound **8** was obtained as a brown liquid in the cooling trap. Simultaneous sublimation of the sodium salt of **5** could not be avoided completely.

Yield: 4.12 g (= ca. 60% crude material); MS (70 eV): m/z (%) = 52(100), 51(73), 50(50), 80(28), 79(16), 49(11).

(c) Pent-*trans*-2-en-4-ynal-diethylacetal **9**. Compound **8** was converted to **9** as described previously [40,41,44]. Purification of **9** was achieved by distillation (K_p^{25} = 27°C). The distillate was dissolved in diethyl ether, filtered over silica gel, and finally purified by TLC.

Yield: 3.15 g (= 41%); TLC (CH–EA, 6:1): R_F = 0.69; GC (OV 101): R_i = 1004; MS (70 eV): m/z (%) = 109(100), 81(37), 44(27), 53(9), 97(7), 125(4), 154(2).

(d) 6-Hydroxy-undec-*trans*-2-en-4-ynal-diethylacetal **11** was prepared via Grignard reaction [44]. Ethylbromide (2.73 g, 25 mmol) dissolved in 15 ml absolute tetrahydrofuran was reacted with 0.61 g (25 mmol) of magnesium filings. This Grignard solution was cooled to −10°C. Pent-2-en-4-ynal-diethylacetal [3.08 g (20 mmol)] dissolved in 20 ml absolute tetrahydrofuran was added dropwise by stirring. The solution was stirred until the ethane gas production ceased. After cooling to −15°C, 2.0 g (20 mmol) hexanal dissolved in 20 ml dry tetrahydrofuran were added dropwise. The mixture was allowed to warm up slowly to 0°C. The solution was stirred for 2 h. Then, still by cooling, 20 ml saturated aqueous NH_4Cl solution and 20 ml diethyl ether were added carefully. The ether layer was separated, the aqueous layer was extracted with diethyl ether. The combined ether extracts were washed with aqueous NH_4Cl solution and dried with K_2CO_3. After filtration the solvent was removed. The residue was dissolved in a mixture of cyclohexane (CH)–ethylacetate (EA) (3:1) and purified by filtration on SiO_2.

Yield: 1.17 g (= 23%); TLC (CH–EA, 3:1): R_F = 0.41; GC (OV 101): R_i = 1761 (TMS-ether); MS (70 eV): m/z (%) = 73(100), 103(17), 255(16), 326(9), 183(7), 281(6), 311(4).

(e) Diethylacetal of 6-hydroxy-*trans,trans*-2,4-undecadienal **12**. Hydrogenation was carried out in analogy to a paper of Esterbauer [42]. 57 mg (1.5 mmol) $LiAlH_4$ were added to a solution of 254 mg (1 mmol) 6-hydroxy-undec-*trans*-2-en-4-ynal-diethylacetal **11** in 3.0 ml dry diethyl ether which was kept at −25°C. The reaction mixture was stirred at this temperature for 3 h. Then within 2 min 1 ml half-concentrated NH_4Cl-solution was added to destroy the excess of $LiAlH_4$. The layers were separated, the aqueous layer was extracted with diethyl ether, the organic solutions were combined. Solvent was removed. Purification was achieved by TLC using cyclohexane–ethylacetate (3:1) as mobile phase.

Yield: 144 mg = (83%); TLC (CH–EA, 3:1): R_F = 0.45; GC (OV 101): R_i = 1783 (TMS-ether); MS (70 eV): m/z (%) = 73(100), 147(78), 282(68), 103(57), 253(26), 257(6), 313(5), 328(0.5).

(f) 6-Hydroxy-*trans,trans*-2,4-undecadienal **3a**. Hydrolysis of the diethylacetal was achieved by treatment with citric acid [45]. 120 mg (0.47 mmol) of raw 6-hydroxy-*trans,trans*-2,4-undecadienal-diethylacetal **12** were dissolved in 0.5 ml 0.1% (w/v) aqueous citric acid and stirred at room temperature for 2 h [45]. Extraction of **3a** was performed with diethyl ether, the ether extract was dried over Na_2SO_4. After filtration the solvent was evaporated in a stream of nitrogen. Purification was achieved by TLC.

Yield: 76 mg (= 89%); TLC (CH–EA, 2:1): R_F = 0.32; GC (OV 101): R_i = 1578/1628 (TMS-ether); MS (70 eV): m/z (%) = 183(100), 73(86), 254(38), 130(14), 143(13), 239(8), 225(4); ^{1}H-NMR (C_6D_6): d = 0.88 (t, J = 7.10 Hz, 3H, -**CH**$_3$), 1.16–1.65 (m, 8H, -(**CH**$_2$)$_4$-), 4.26–4.29 (m, 1H, -**CH**(OH)-), 6.15 (dd, J = 15.34, 7.90 Hz, 1H, -CH = **CH**-CH = O), 6.24 (dd, J = 15.29, 5.68 Hz, 1H, -CH(OH)-**CH** = CH-), 6.50 (dd, J = 15.29, 10.92 Hz, 1H, -CH(OH)-CH = **CH**-), 6.79 (dd, J = 15.34, 10.92, 1H, -**CH** = CH-CH = O), 9.56 (d, J = 7.92, 1H, -**CH** = O)

2.3. Autoxidation

An amount of 20 mg fatty acid (linoleic acid or oleic acid, respectively) was dissolved in 30 ml 0.1 *M* Tris (pH 7.4) and 60 ml 0.15 *M* KCl. Autoxidation was started by addition of 5 ml 20 m*M* ascorbate and 5 ml 0.8 *M* $FeSO_4$, following the well elaborated LPO method worked out by Esterbauer [24]. The solutions were incubated at room temperature. Reaction time was 24 h in

case of linoleic acid and five days in case of oleic acid.

2.4. Preparation of samples for GC–MS

After the indicated incubation time 10 ml 0.05 *M* methanolic pentafluorobenzyl (PFB) hydroxylamine and 35 mg 1-hydroxy-2-dodecanone (internal standard) were added and PFBO derivatization was performed according to methods of Van Kuijk et al. [36] and Loidl-Stahlhofen et al. [27,28]. The PFBO derivatives were extracted three times with cyclohexane–diethyl ether (4:1). Subsequent treatment with diazomethane converted fatty acids into their methyl esters. Subfractionation of lipid peroxidation products after PFBO derivatization was achieved by TLC in cyclohexane–ethyl acetate (3:1). The fraction containing PFB oxime derivatives of α-hydroxyaldehydes (and simultaneously 4-hydroxy-2-alkenals) (fraction A, $R_F = 0.48–0.59$) and the fraction containing derivatized 6-hydroxy-2,4-alkadienals, (fraction B, $R_F = 0.40–0.47$) were spiked with external standards (O-PFB oxime derivative of 2-hydroxyundecanal and 6-hydroxy-2,4-undecadienal, respectively). Trimethylsilylation was performed as described previously [27]. Analysis of fraction B via GC–MS in case of oleic acid resulted in detection of 4-hydroxyalkanals as previously unknown lipid peroxidation products of oleic acid.

2.5. Gas chromatography–mass spectrometry

GC was carried out with a Fisons (Mainz-Kastel, Germany) HRGC 5160 Mega Series chromatograph equipped with a flame ionization detector, using a DB-1 fused-silica glass capillary column (30 m × 0.32 mm I.D., 0.1 μm film thickness), temperature programmed from 80 to 280°C at 3°C min^{-1}. The temperature of the injector and detector were kept at 270 and 290°C, respectively. The carrier gas was hydrogen and the splitting ratio was 1:30 (80%). Peak-area integration was achieved with a Merck D-2500 integrator.

GC–MS was performed on a Finnigan MAT 312 mass spectrometer connected to a MAT-SS-300 data system. EI mass spectra were recorded at an ionization energy of 70 eV. A Varian 3700 gas chromatograph with a 30 m × 0.3 mm I.D. A DB-1 fused-silica column was used for sample separation. The carrier gas was hydrogen and the temperature programme was the same as used for GC.

3. Results

Recently we reported on the detection of short-chain α-hydroxyaldehydes in biological material [26,46]. Later we have been able to present evidence that these 2-hydroxyalkanals are produced during lipid peroxidation of polyunsaturated fatty acids (PUFAs) [27,28]. Based on the GC–MS analysis of volatile and non-volatile secondary autoxidation products a general scheme for the generation of di- and polyoxygenated LPO products was developed [28] (Fig. 1).

Key step in the autocatalytic oxidation of hydroperoxides of unsaturated fatty acids (LOOHs) is the activation of C – H bonds adjacent to the diene system causing a preferential abstraction of a hydrogen radical: it is possible to write several mesomeric structures (**a–c**) of these radicals. These radicals tend to react with oxygen. In a final step abstraction of a hydrogen occurs, resulting in the production of different bishydroperoxides **d–f**. Decomposition of **d** and **e** yields α-hydroxyaldehydes **1** and 4-hydroxy-2-alkenals **2**, respectively [35]. Analogous reaction of **f** should result in formation of 2-hydroxy-2,4-alkadienals **3**, previously unknown LPO products. Such highly conjugated compounds seem to be prone to further oxidation. Therefore we assumed that **3** would occur in the oxidation product mixtures only in traces.

Knowledge of the chromatographic behaviour was assumed to be necessary for the detection of **3** in the complex reaction mixture. Consequently 6-hydroxy-2,4-undecadienal **3a** was synthesized (Fig. 2).

First furfural **4** was transformed according to the procedure of Foster and Agosta [41] to its tosylhydrazone **5**. The tosylhydrazone **5** was

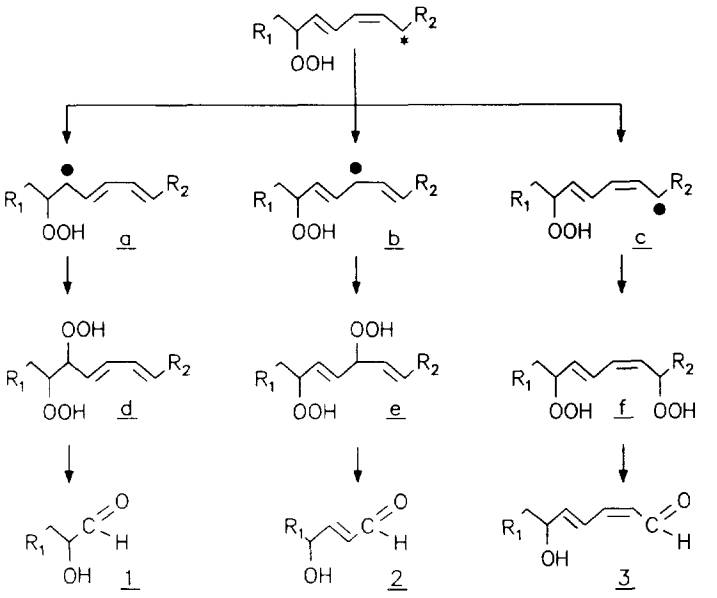

Fig. 1. Mechanistic pathway for dioxygenation during lipid peroxidation of linoleic acid. R_1, R_2: alkyl or acidic end of LOOH, respectively.

converted with NaH to its sodium salt which was decomposed by oxidation to furyldiazomethane **6** [42]. This unstable intermediate **6** reacted to the also instable carbene **7** which was further decomposed to pent-2-en-4-ynal **8** [43]. Compound **8** was converted via orthoformic acid triethylate to its acetal **9** [44]. Compound **9** was transformed with ethylmagnesiumbromide to the Grignard compound **10** which was reacted with hexanal to 6-hydroxy-undec-2-en-4-ynaldiethylacetal **11** [44]. Compound **11** was reduced with $LiAlH_4$ to the 6-hydroxy-2,4-undecadienal-diethylacetal **12** which was subjected to cleavage by action with 0.1% (w/v) citric acid to the requested 6-hydroxy-2,4-undecadienal **3a** [45]. Compound **3a** was characterized by ^{1}H-NMR, GC and GC–MS.

The EI-mass spectrum of the PFB-oxime TMS-ether derivative of 2-hydroxy-2,4-undecadienal is shown in Fig. 3.

In addition to the molecular ion at $m/z = 449$, key ions at $m/z = 434$ (M − 15), $m/z = 378$ (α-cleavage at the C-C bond adjacent to the trimethylsilyloxy function) and $m/z = 252$ (loss of pentafluorobenzyloxy group) confirmed the structural identity of **3a** after PFBO and MSTFA derivatization.

In a subsequent step the PFB oxime derivative of **3a** was used as external standard to enrich 6-hydroxy-2,4-alkadienals formed after lipid peroxidation of linoleic acid. The GC after subfractionation, PFBO derivatization and TLC showed the presence of a complex mixture of lipid peroxidation products (Fig. 4a).

Nevertheless the O-PFB oxime TMS-ether derivative of 6-hydroxy-2,4-undecadienal **3a** was identified unambiguously using the key ion at $m/z = 181$ (indicating a PFBO derivative [32]), and those at $m/z = 378$ and 252 to detect **3a** (Fig. 3, Fig. 4b).

Thus 6-hydroxy-2,4-alkadienals could be shown to be typical autoxidation products of the $(n - 6)$ fatty acid linoleic acid. This finding is another valuable hint that the production of dioxygenated PUFAs during the autoxidation processes proceeds via the postulated reaction mechanism (Fig. 1).

3.1. Lipid peroxidation of oleic acid

In addition, this mechanistic concept proved its general utility by its application to other model systems [34,45].

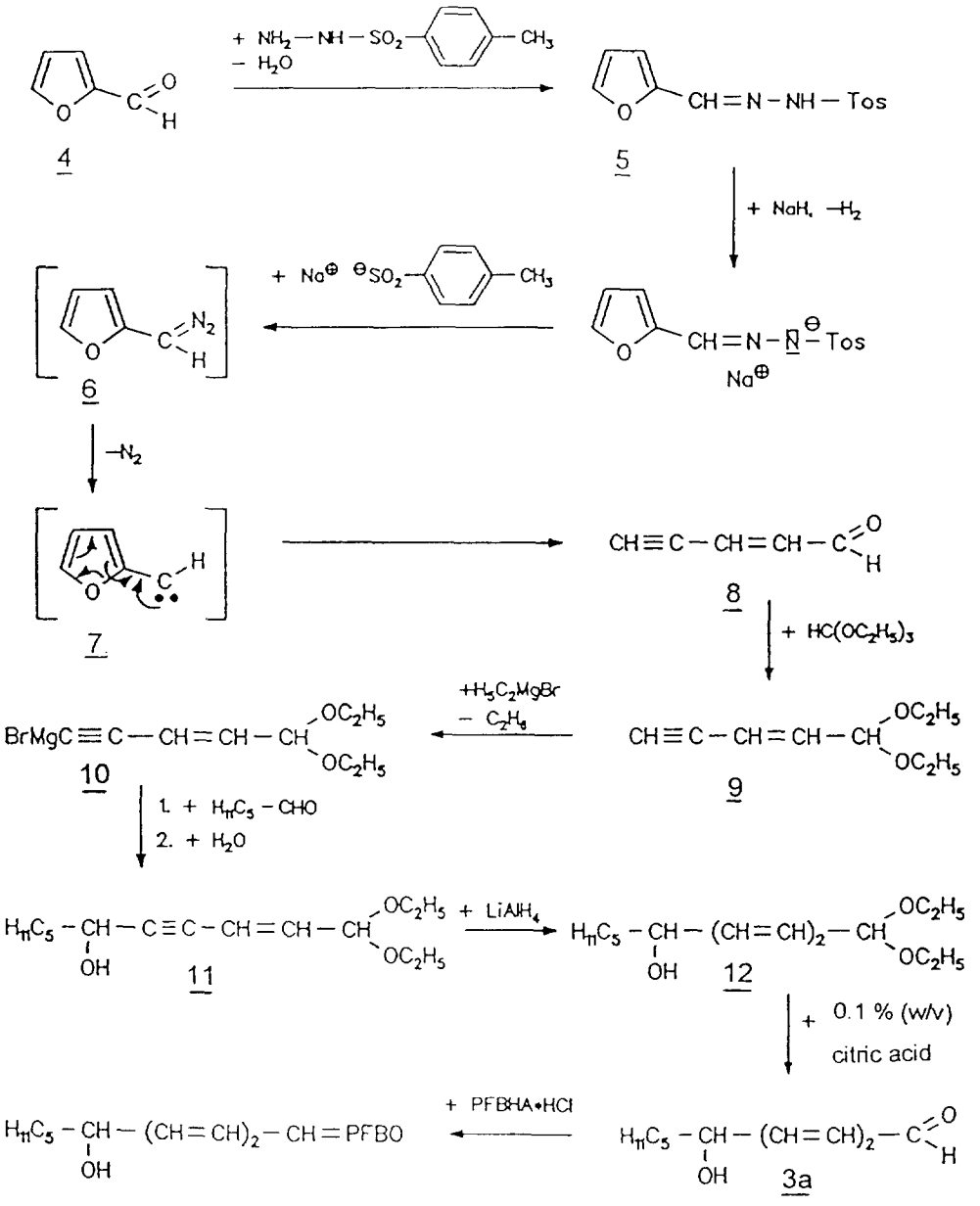

Fig. 2. Synthesis of 6-hydroxy-2,4-undecadienal **3a**.

In case of oleic acid the existence of 2-hydroxyaldehydes with to 8- (**1a**), 9- (**1b**), 10- (**1c**), and 11-C-atoms (**1d**) could be demonstrated [27]

The yield of these aldehydes is about only 1/1000 compared to the main α-hydroxyaldehydes 2-hydroxyheptanal (autoxidation product of $(n-6)$ fatty acids [27]) and 2-hydroxybutanal (autoxidation product of $(n-3)$ fatty acids [47]). This reflects the earlier reported much higher stability of oleic acid against oxidation, since formation of primary hydroperoxides is not favoured by a pentadienyl system [48].

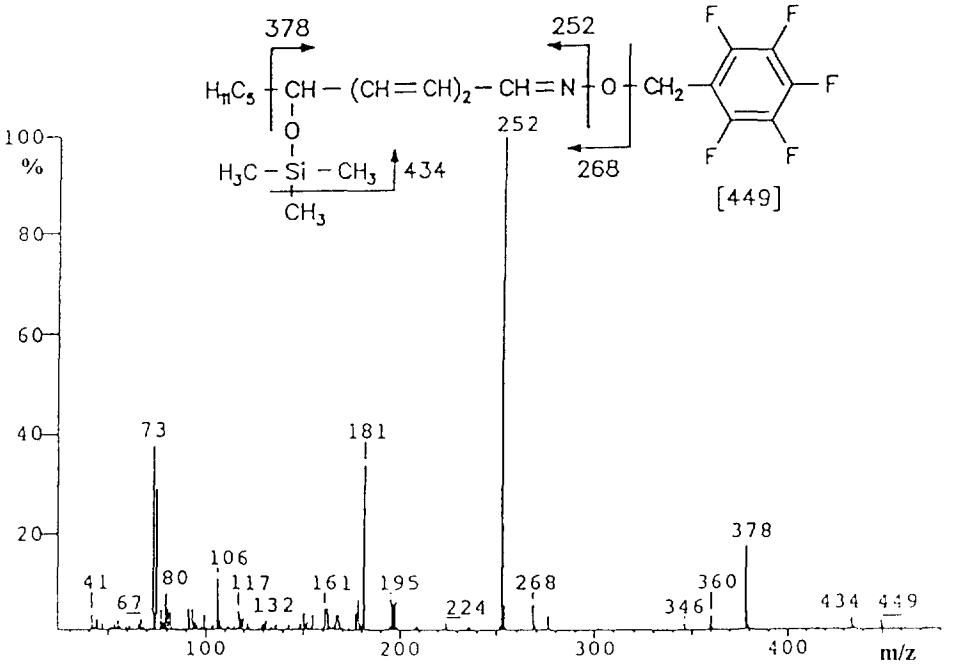

Fig. 3. EI-MS of the O-PFB oxime TMS-ether derivative of 6-hydroxy-2,4-undecadienal **3a**.

In oleic acid the CH_2 groups adjacent to the double bond are activated (in Fig. 5 marked with an asterisk). Taking into account all equivalent mesomeric structures of the primary radicals, four primary hydroperoxides (8-, 9-, 10- and 11-oleic acid hydroperoxides OAOOH) can be produced (Fig. 5) [11,13,14].

The latter may suffer analogous dioxygenation. Secondary abstraction of an hydrogen radical may proceed in allylic position of these hydroperoxides or be induced by the neighbourhood of the hydroperoxy function (in Fig. 5 marked with an asterisk). Fig. 6 shows the mechanistic explanation for the generation of 2-hydroxyoctanal **1a**, 2-hydroxynonanal **1b** and 2-hydroxydecanal **1c** representing secondary oxidation products of 10-hydroperoxy-8-octadecenoic acid (10-OAOOH) and 11-hydroperoxy-9-octadecenoic acid (11-OAOOH), respectively. More difficult is the explanation for formation of 2-hydroxyundecanal **1d**. We assume an additional isomerization of the double bond in the primarily produced radicals.

Dioxygenation of OAOOHs may also proceed via an intramolecular mechanism [48,49]. Secondary abstraction occurs by hydrogen shift from the activated allylic position to the radical site of the oxygen in the primary alkoxyradical producing hydroxyhydroperoxides (Fig. 7).

In fact traces of 4-hydroxy-2-alkenals **2** were detected as PFB oxime TMS-ether derivatives via GC–MS using appropriate ion traces (Table 1, Fig. 8).

Since PFB oximes exist as *syn* and *anti* isomers, the presence of a sufficient amount of derivatized 4-hydroxy-2-alkenals **2** is indicated by double peaks in the recorded ion current (RIC).

4-Hydroxy-2-decenal **2a** can be generated by secondary oxidation of 9-hydroperoxy-10-octadecenoic acid (9-OAOOH) (Fig. 9). In fact 12-oxo-9-hydroxy-10-dodecenoic acid **13**, containing the acidic end of the original 9-OAOOH, can also be detected [38]. 4-Hydroxy-2-undecenal **2b** may be formed by further oxidation of 8-hydroperoxy-9-octadecenoic acid (8-OAOOH) or 11-OAOOH. Oxidative degradation of 10-OAOOH explains the detection of 4-hydroxy-2-dodecenal **2c** as outlined in Fig. 9. Activated positions in primary hydroperoxides, prone to further oxidation, are marked with an asterisk.

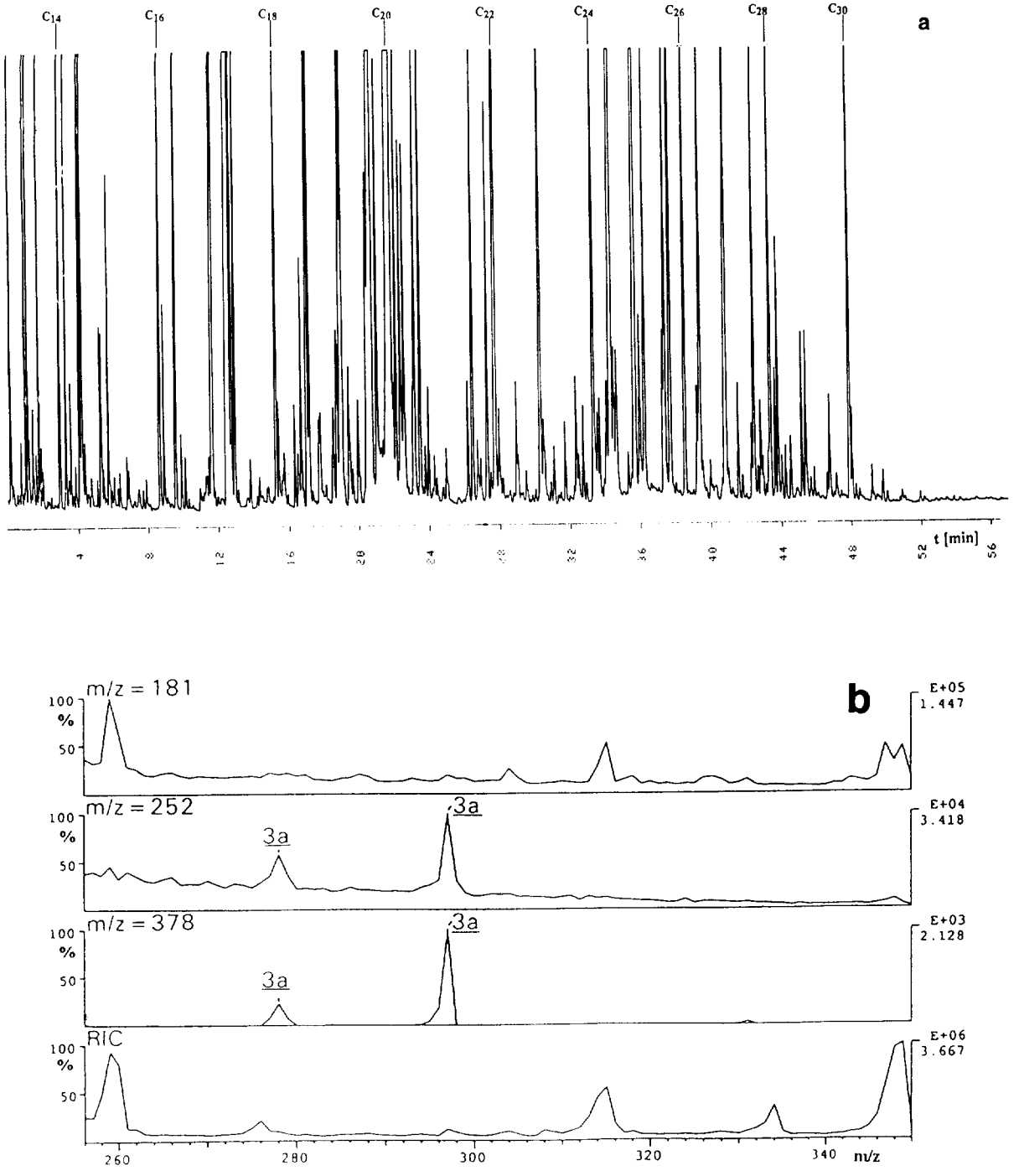

Fig. 4. (a) GC of LPO products of linoleic acid after TLC subfractionation (O-PFB oxime TMS-ether derivatives). (b) Total ion current and EI-MS screening procedure for 6-hydroxy-2,4-alkadienals **3** (O-PFB oxime TMS-ether derivatives).

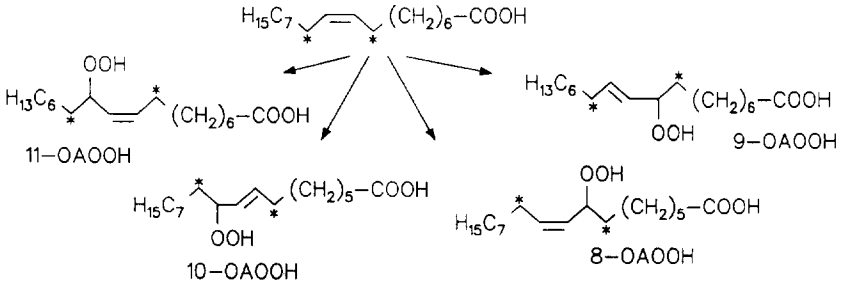

Fig. 5. Generation of 8-, 9-, 10- and 11-oleic acid hydroperoxides.

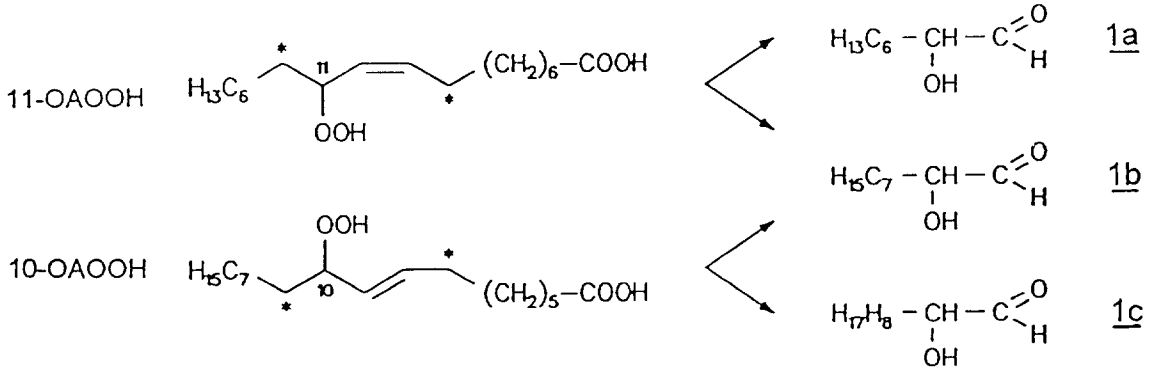

Fig. 6. Mechanistic explanation for the generation of 2-hydroxy-octanal **1a**, 2-hydroxynonanal **1b** and 2-hydroxydecanal **1c** during lipid peroxidation of oleic acid.

Consequent analysis of lipid peroxidation products of oleic acid after PFBO derivatization revealed the previously unknown existence of 4-hydroxyalkanals **14**. 4-Hydroxyheptanal **14a**, 4-hydroxyoctanal **14b** and 4-hydroxynonanal **14c** were identified as typical secondary lipid peroxidation products. The specific screening procedure for this unknown class of autoxidation products and the characteristic EI-mass spectrum of 4-hydroxynonanal **14c** as O-PFB oxime TMS-ether derivative are presented in Figs. 10 and 11, respectively.

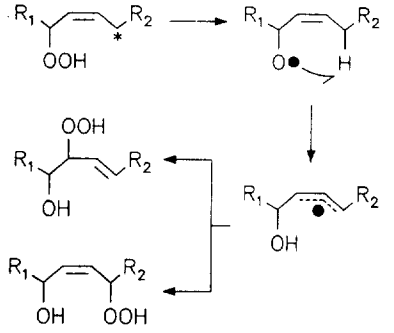

Fig. 7. Alternative mechanistic pathway for dioxygenation during lipid peroxidation of oleic acid.

The molecular mass of the compounds can be deduced from the fragment at $[M-15]^+$. Other key ions result from α-cleavage reactions at the trimethylsilylated oxygen yielding fragments at $m/z = 354$ and $m/z = 173$. In addition, traces of 9-oxo-6-hydroxynonanoic acid **15** representing a corresponding $(n-4)$-hydroxy-n-oxo acid could be detected as O-PFB oxime TMS-ether TMS-ester derivative.

4. Discussion

Secondary oxidation of primary formed hydroperoxides in the course of lipid peroxidation seems to be a reaction of general importance. A

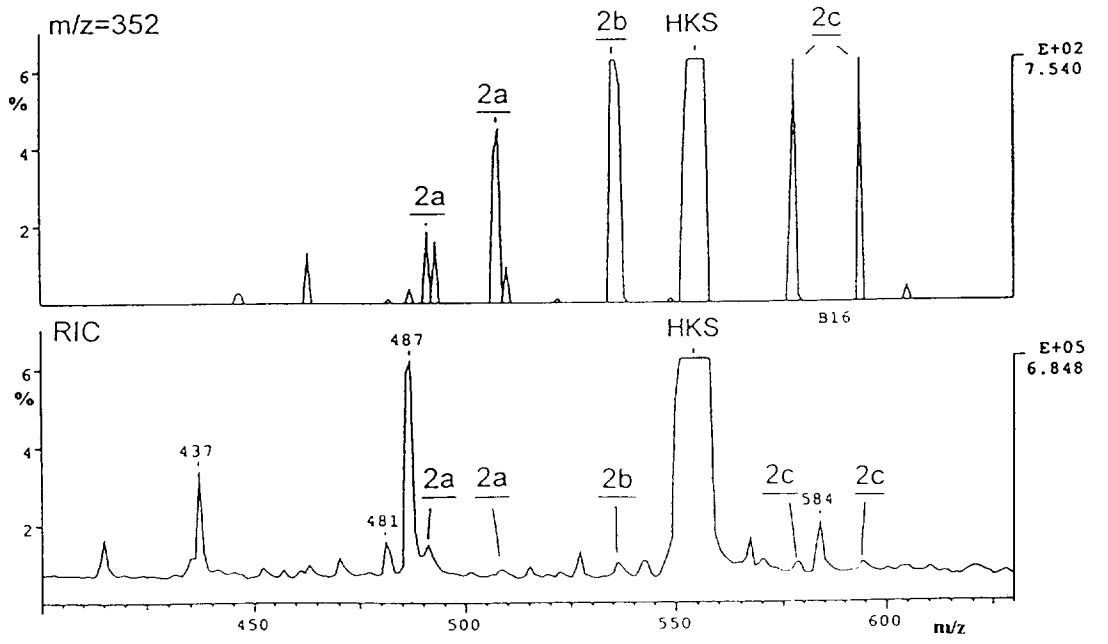

Fig. 8. Total ion current and EI-MS screening procedure for 4-hydroxy-2-alkenals **2** (O-PFB oxime TMS-ether derivatives). HKS = hydroxyketone (internal standard).

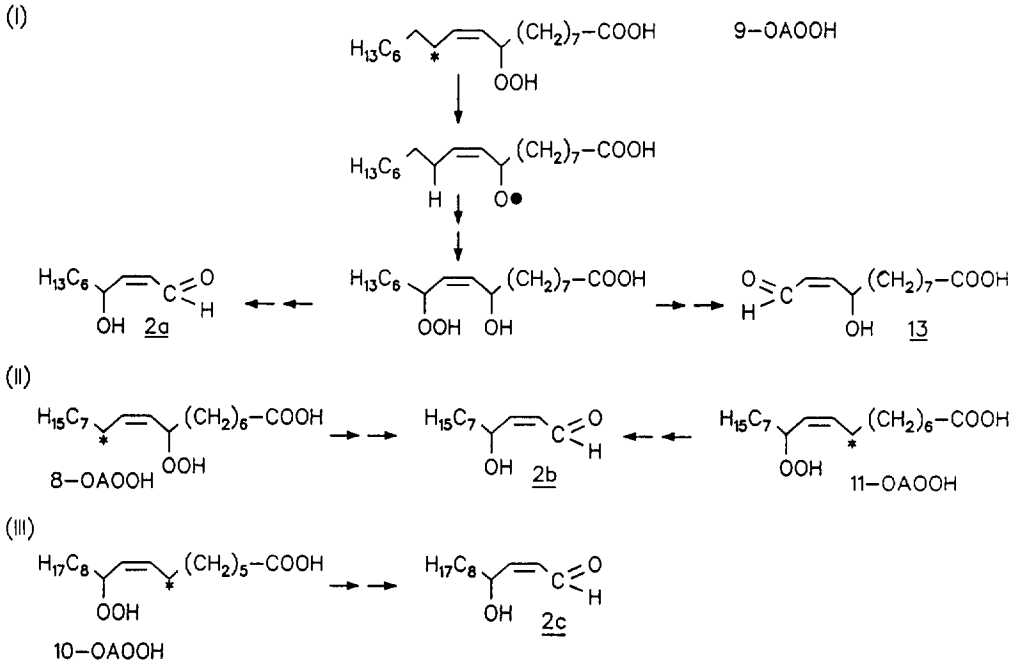

Fig. 9. Mechanistic explanation for the generation of 4-hydroxy-2-decenal **2a**, 4-hydroxy-2-undecenal **2b** and 4-hydroxy-2-dodecenal **2c** during lipid peroxidation of oleic acid.

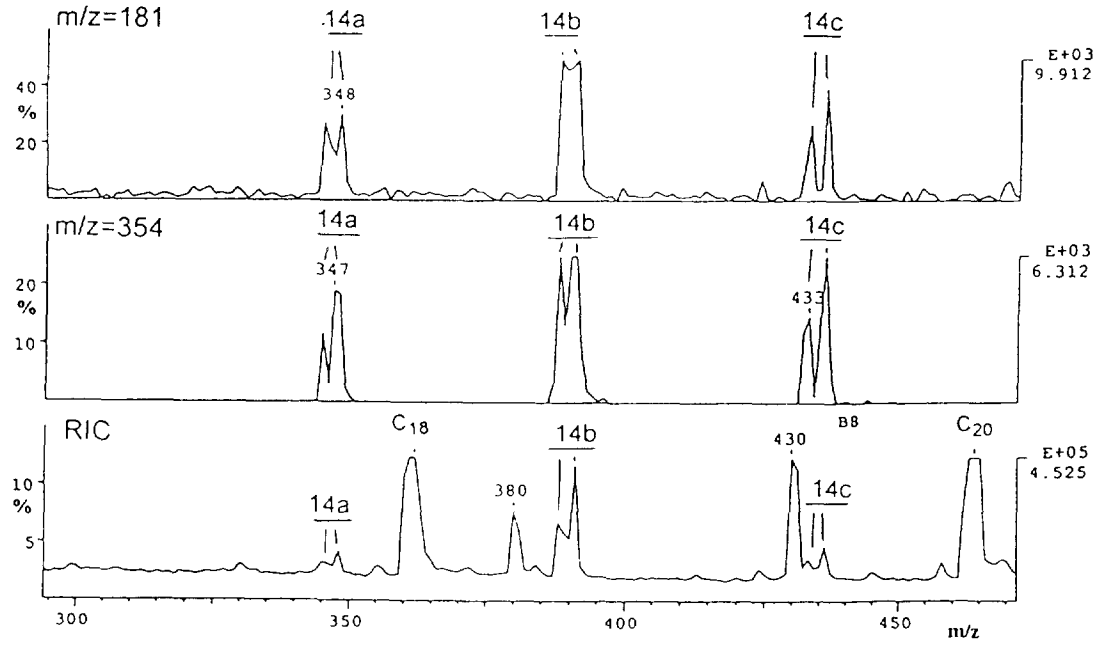

Fig. 10. Total ion current and EI-MS screening procedure for 4-hydroxyalkanals **14** (O-PFB oxime TMS-ether derivatives).

number of non-volatile secondary autoxidation products (dioxygenated fatty acids) have been identified after LPO of PUFAs [50–54].

The presented mechanistic concept of dioxygenation does not only explain the formation of non-volatile LPO products and volatile α-hy-

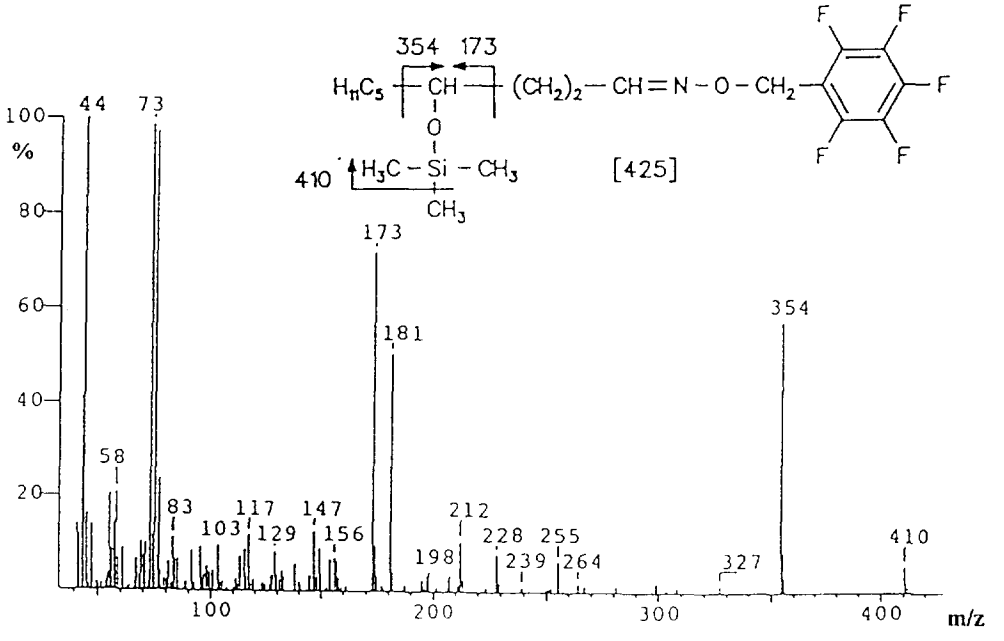

Fig. 11. EI-MS of the O-PFB oxime TMS-ether derivative of 4-hydroxynonanal **14c**.

droxyaldehydic compounds in the course of lipid peroxidation, it enabled us also to predict the occurrence of 6-hydroxy-2,4-alkadienals as autoxidation products of linoleic acid.

The existence of 4-hydroxy-2-alkenals **2** after autoxidation of oleic acid provides additional supporting evidence for the proposed general dioxygenation mechanism. 4-Hydroxyalkanals **14** represent a further class of previously unknown dioxygenated LPO products of oleic acid. We assume that these compounds were generated from α-hydroxyaldehydes by loss of water and further attack of the activated CH_2-groups in the thus produced α,β-unsaturated aldehydes by oxygen.

The relevance of aldehydic lipid peroxidation products concerning the deleterious effects of oxidative stress remains unclear. Further investigations have to show if and how they are involved in destruction of membrane structure and various impairments of enzyme conformation and activity. It cannot be excluded that certain hydroxyaldehydic compounds, for example 6-hydroxy-2,4-alkadienals **3** or certain α-hydroxyaldehydes **1**, represent second toxic messengers as already proposed for the vinylogous 4-hydroxy-2-alkenals, e.g. 4-HNE [16,55].

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