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Reinvestigation of lipid peroxidation of linolenic acid ☆

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Abstract

Recently, we deduced a mechanism for lipid peroxidation of linoleic acid [1]. This mechanism was now applied to predict the occurrence of previously unknown lipid peroxidation products of linolenic acid. The proposed structures of peroxidation products allowed to search for these predicted compounds in reaction mixtures with the aid of 'ion trace' by mass spectrometry. Thus, a great number of previously unknown lipid peroxidation products was detected. It is assumed that these compounds also occur – at least as intermediates – in lipid peroxidation processes in mammalian tissue.

Key words: Keywords: Lipid peroxidation; Linolenic acid; α-Hydroxyaldehyde; Glyoxal; Hydroxy-oxo-octadecadienoic acid; Dihydroxyoctadecadienoic acid; ω-Oxo(ω-1)hydroxy fatty acid

1. Introduction

Recently, we explained the occurrence of dioxygenated products originating from oxidation of linoleic acid with oxygen in the presence of Fe^{2+}/ascorbate [1] by considering the possibility that in a hydroxyperoxid of an unsaturated acid there are activated hydrogens which can be removed as easily as hydrogens in the activated double allylic position 11 of the starting molecule, linoleic acid [1].

The situation in linolenic acid 1 is even more complicated, since linolenic acid 1 already contains 2 activated allylic CH_2 groups in position 11 and 14 (Scheme 1).

If linolenic acid is subjected to the conditions of lipid peroxidation, e.g., by treatment with Fe/ascorbate in the presence of oxygen [2,3] 4 regioisomeric hydroperoxides 2, 3, 4 and 5 are obtained, although in different yield: hydroperoxides with the oxygen function at position 9 or 16 predominate [4–6].

2, 3, 4 and 5 react in similar way as the corresponding hydroperoxides of linoleic acid to a bouquet of products in presence of iron ions by cleavage of the oxygen-oxygen-bond. So for instance, 16-hydroperoxy-14-*trans*-9,12-*cis*-octadecatrienoic acid 2 (Scheme 2) produces the radical 6. This radical 6 can react either by abstraction of a hydrogen radical from another molecule with an activated C-H bond (e.g., at carbon 11 or 14 of a second molecule of linolenic acid) producing a hydroxy compound 13 or by cleavage of an adjacent C-C bond. In this case, an aldehyde (7 or 10) and a hydrocarbon radical is produced (e.g., 8 or 11) [7]. The latter are stabilized by hydrogen abstraction from another molecule, forming 9 or 12. Thus, a typical decomposition product of lipid peroxidation of linolenic acid is ethane 9 [8–10] (Scheme 2).

Alternatively, we also expect that the radical 6 may loose the activated hydrogen adjacent to the oxygen radical-site producing the oxo acid 14 [11] or – if the adjacent double bond is attacked – an epoxy allylic radical 15 resp. 16 [7,12,13]. A second molecule of oxygen may react at the radical sites of 15 or 16. Thus, the formation of the stabilized hydroperoxyepoxy acids 17 or 19 is expected. These may decompose again by action of iron ions to the alkoxy radicals which abstract

Abbreviations: LPO, lipid peroxidation; PUF, polyunsaturated fatty acid; PFBO, pentafluorobenzyloxime; PFBHA, pentafluorobenzylhydroxylamine; MSTFA, *N*-methyltrimethylsilyltrifluoro-acetamide; LOOH, hydroperoxy linolenic acid; LOH, hydroxy linolenic acid.

☆ Dedicated to Professor Dr. Wolfgang Lüttke on the occasion of his 75th birthday.

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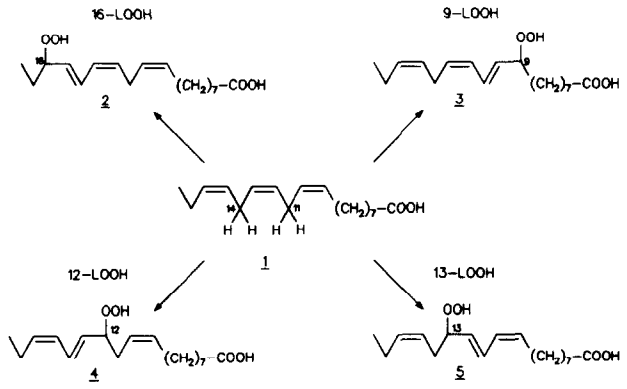


Scheme 1.

from another molecule a hydrogen atom, producing the hydroxyepoxy acids 18 resp. 20.

The other hydroperoxides 3, 4 and 5 decompose in similar manner.

As a result a great number of hydroxy acids, oxo acids, epoxy acids and epoxy-hydroxy acids were detected as products of lipid peroxidation of linolenic acid [11–14].

In addition, hydroperoxides, hydroperoxy-epoxides and hydroperoxy-bicyloendoperoxides were identified from oxidized linolenate not only applying autoxidation conditions [14], but also by photosensitized oxidation [15,16] or ionizing irradiation [17]. It was suggested that cyclic peroxide-hydroperoxides are produced by cyclization of 4 and 5 [14]. In the presence of enzymes oxidation of linolenic acid, α- and γ-ketol formation was also observed [18–21].

2 or the derived hydroxy derivative 13 contains two activated C-H bonds in position 11, resp. 16, prone for hydrogen abstraction from another radical formed in the reaction process. As a consequence the radicals 21 and 22 should be produced in the reaction medium. The radicals 21 and 22 can also be described in the mesomeric forms 23 and 24 resp. 25 and 26 (Scheme 3).

Since these radicals can add oxygen as well as a radical formed by hydrogen abstraction from 1, a great number of dioxygenated products and the derived compounds is expected to be produced. For instance, the radical 22 can add one molecule of oxygen, the new radical abstracts from somewhere a hydrogen, the thus formed 16-hydroperoxy-11-hydroperoxy-9,12,14-octadecatrienoic acid is decomposed by cleavage of the oxygen-oxygen bond and the obtained radical is finally stabilized by hydrogen abstraction to 27. Similar reactions would explain the formation of 28, 29, 30, 33 etc.

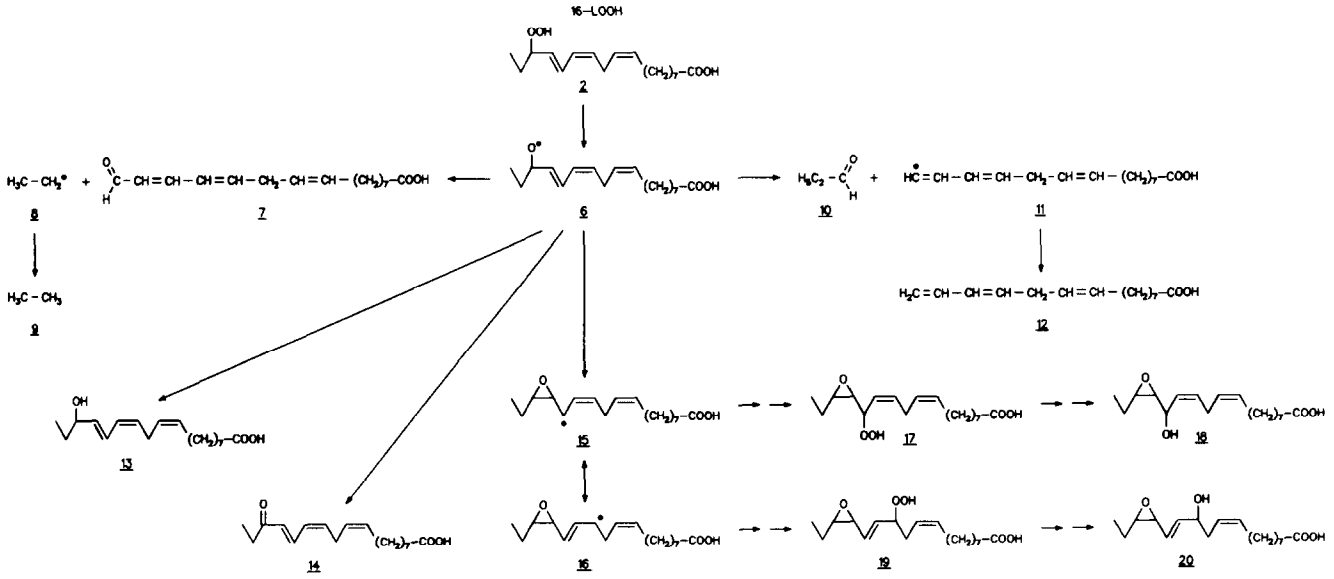
Further reactions – exemplified with 2 as starting material – lead to aldehydes represented in Schemes 4, 5 and 6. These can easily be detected after appropriate derivatization by GC-MS.

Similar reactions are expected to occur with the other hydroperoxides 3, 4 and 5. This paper describes the identification of such dioxygenated compounds obtained by oxidation of linolenic acid.

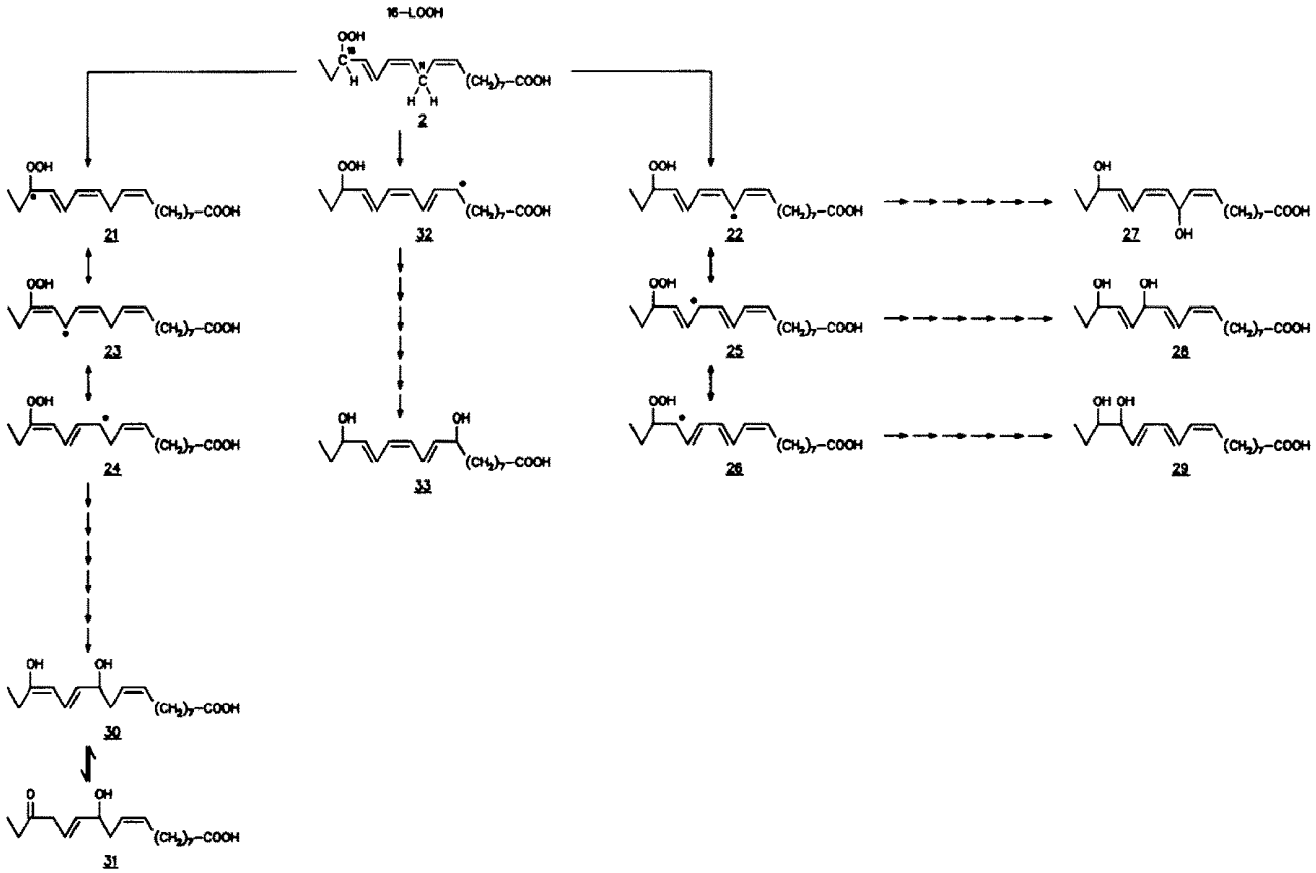
2. Materials and methods

2.1. Materials

N-Methyltrimethylsilyltrifluoracetamide (MSTFA) was obtained from Macherey & Nagel (Düren). All

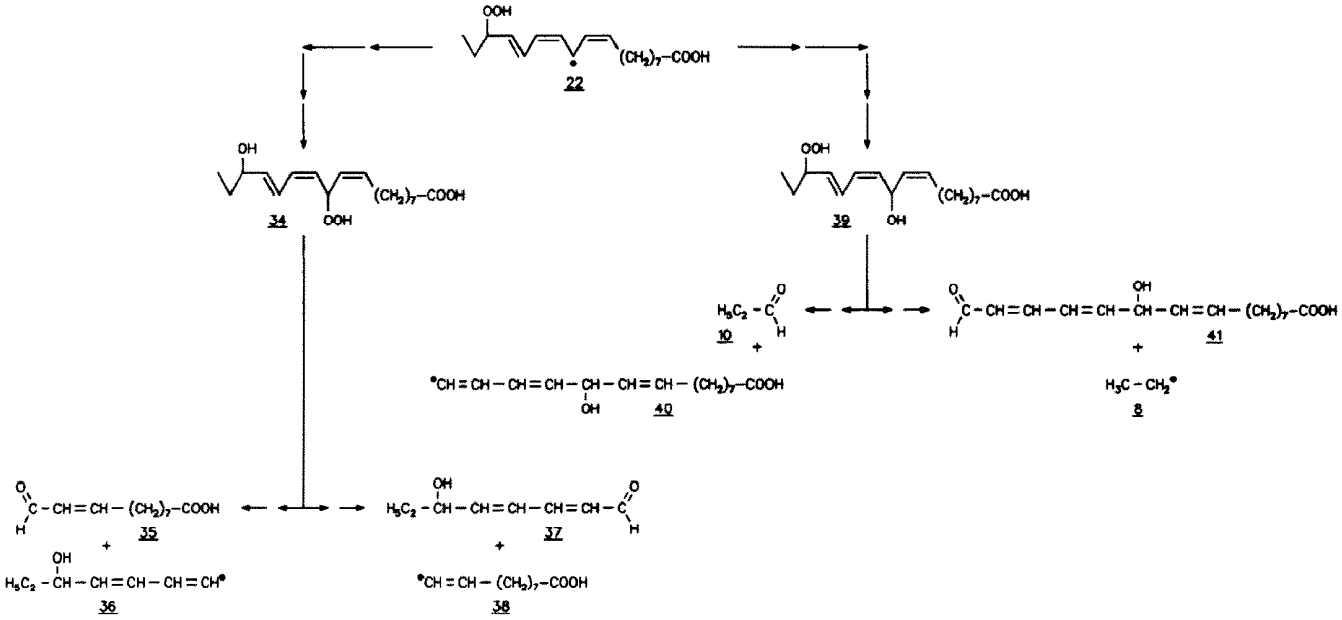


Scheme 2.

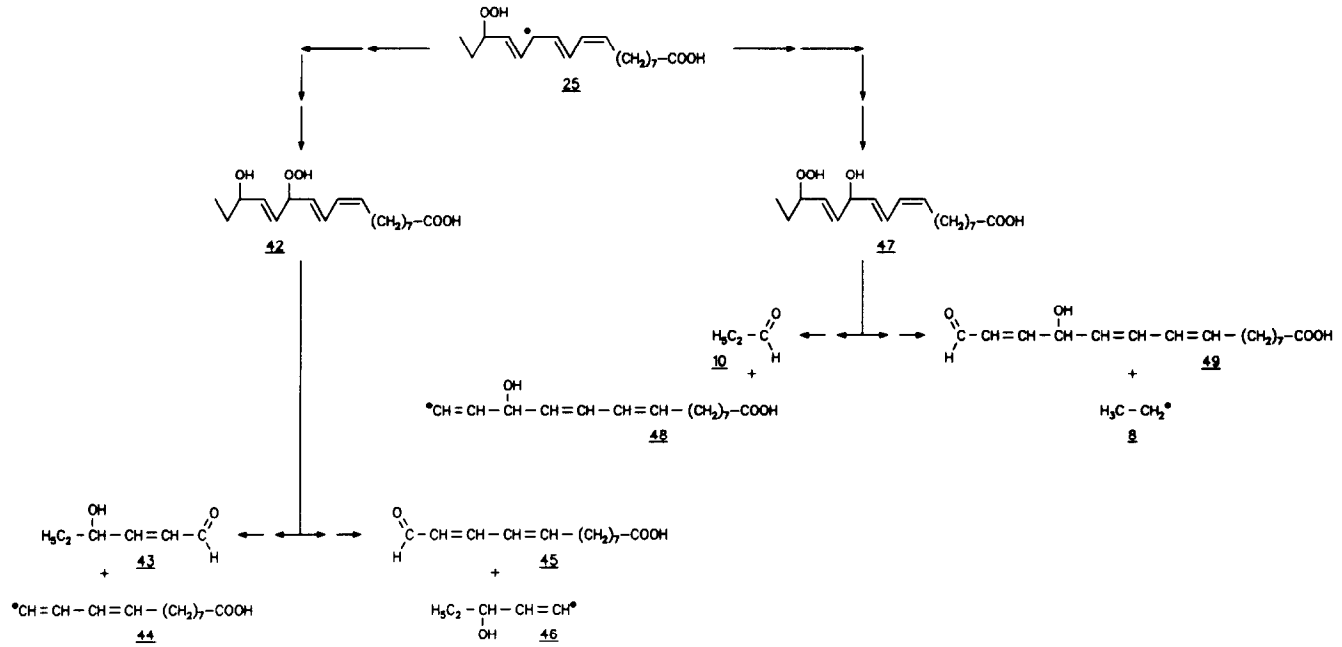


Scheme 3.

other chemicals were purchased from Fluka (Neu Ulm, Germany). Solvents, obtained from Merck (Darmstadt, Germany) were destilled before use. TLC was performed with home made 0.75 mm PF_{254} silica-gel 60 (Merck, Darmstadt) plates. The linolenic acid was stored at −18°C under argon.



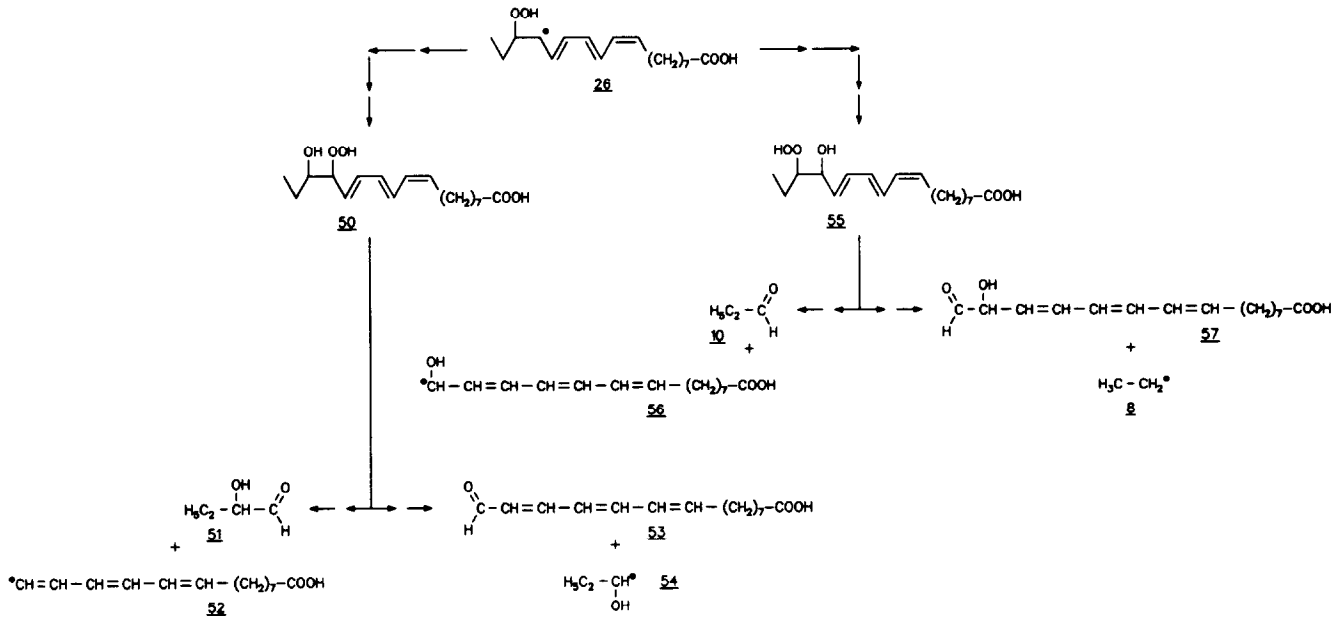
Scheme 4.



Scheme 5.

2.2. Preparation of the standard compounds, 9,10-, 12,13-, 15,16-dihydroxyoctadecadienoic methylates

Epoxidation of linolenic acid in presence of 3-chloroperbenzoic acid as described previously [22,23] provided a mixture of mono-, di- and triepoxyoctadecadienoic acids. This mixture was treated with an etheric diazomethane solution to convert the acids in their methylates. The mixture of mono-, di- and triepoxyoctadecadienoic methylates was subjected to TLC in cyclohexane/ethyl acetate 3:1 in order to separate the fraction containing the monoepoxyoctadecadienoic acids. This fraction (R_f = 0.64–0.82) containing a mixture of 9,10-, 12,13-, 15,16-epoxyoctadecadienoic meth-



Scheme 6.

ylates was hydrolyzed using the Nafion catalyst NR50-H^+-form [24] resulting in a mixture of 9,10-, 12,13- and 15,16-dihydroxyoctadecadienoic methylates.

2.3. Autoxidation

Autoxidation was performed as described previously [25] following the well elaborated lipid peroxidation method worked out by Esterbauer [26]. The solution was incubated at room temperature up to 2 days. The carbonyl groups of LPO products were transformed to pentafluorobenzyloxime derivatives, trimethylsilylated with MSTFA and identified with GC-MS by EI mass spectrometry. The aldehydes were also investigated by formation of MSTFA adducts as described previously [25].

2.4. Reduction with $NaBH_4$ and Rh/H_2

After oxidative degradation of linolenic acid (about 48 h incubation time) the solution was extracted with chloroform. After removal of the solvent the fatty acids were converted into their methyl esters by treatment with an etheric diazomethane solution. The methylates were subjected to reduction with $NaBH_4$ [1,27]. The dihydroxyoctadecadienoic methylates were separated from other products by TLC in cyclohexane/ethyl acetate 1:1. The fraction containing the 9,13- and 12,16-dihydroxyoctadecadienoic methylates ($R_f = 0.53–0.73$) was detected by co-chromatography of the standard mixture of synthesized 9,10-, 12,13- and 15,16-dihydroxyoctadecadienoic methylates at the brink of the plates. Detection was performed with 10% ethanolic $H_3[P(MO_3O_{10})_4]$ and heating [28].

The fraction of dihydroxyoctadecadienoic methylates obtained after TLC chromatography was subjected to reduction with Rh/H_2 on Al_2O_3 as described previously [1,29].

2.5. Gas chromatography-mass spectrometry

GC was carried out with a Carlo Erba 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.), temperature programmed from 80 to 280°C at 3°C/min. 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. 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. 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

As described in the introduction the involvement of different radicals in lipid peroxidation of linolenic acid and the decomposition of the various hydroperoxides 2, 3, 4 and 5 in many directions as outlined for 2 in Schemes 2–6 results in the formation of a huge number of primary, secondary and tertiary reaction products. As a consequence complex mixtures must be separated. This requires the application of a combination of different chromatographic steps. Since some of the expected products show great reactivity, e.g., hydroxyaldehydes are prone to undergo dimerisation, derivatisation is required at an early stage in the working up procedure.

Therefore, the reaction mixture was treated immediately after interruption of the oxidation with pentafluorobenzylhydroxylanine in order to protect the aldehyde group. Next the reaction mixture was extracted and reacted with diazomethane to convert acids into their methylates. The thus obtained mixture was subjected to reduction with $NaBH_4$ to convert hydroperoxy groups into hydroxy functions (compounds with hydroperoxy functions decompose even as trimethylsilylderivatives in the GC). The thus obtained mixture was separated by TLC. Standard compounds were used to recognize the zones of different polarity. The zones were scratched off and eluted. The obtained fractions were treated, if necessary, with MSTFA to trimethylsilylate hydroxy groups.

Since trimethylsilyl derivatives of unsaturated hydroxy fatty acid methylates often do not show peaks which allow the deduction of the position of the original hydroxy function in the chain, fractions containing unsaturated hydroxy acid methylates were hydrogenated with Rh/H_2 [29] to convert them into saturated derivatives. After trimethylsilylation the mixtures were subjected to analysis by GC-MS. Mass spectra of the thus obtained saturated trimethylsilylated hydroxy acid methylates allow an unambigous identification of the position of the original OH-groups [30].

Each fraction was separated by GC-MS. Since the products were expected to occur only in tiny amounts burried under different products a search for key ions typical for expected groups was carried out using ion traces. So for instance pentafluorobenzyloxime spectra show a typical fragment of mass 181 [31], those of aldehyde MSTFA-adducts typical ions at masses 110, 134, 184, 228 [32], those of trimethylsilylated hydroxy acids are characterized by fragments of masses 73, 75, 89, 103, 129 [33,34].

The hydroxyaldehydes were determined as PFB

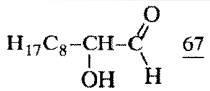
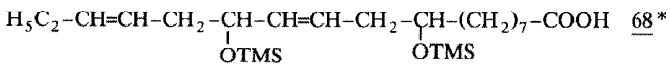
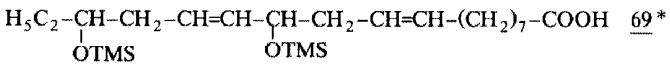
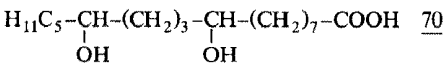
Table 1
Data of all detected LPO-products of linolenic acid

Compound	Derivative	Molecular weight	*RI* value	Key fragments (m/z (%))	Literature
$H_5C_2-C(=O)-H$ 10	MSTFA-adduct	257	1040	257 (1, M^+), 242 (3, M^+-15), 228 (100), 131 (16, M^+-126), 184 (56), 134 (26), 110 (11)	35–39
$H_5C_2-CH=CH-CH=CH-C(=O)-H$ 58	MSTFA-adduct	309	1393 isomeres 1430	309 (21, M^+), 294 (6, M^+-15), 280 (10, M^+-29), 228 (1), 184 (29),	36,39–41
	PFBO-derivative	305	1655 isomeres 1664 1675	183 (15,M^+-126), 110 (15), 305 (13, M^+), 276 (51, M^+-29), 181 (100), 124 (7, M^+-181)	
$H_5C_2-CH=CH-C(=O)-H$ 59	MSTFA-adduct	283	1290	283 (1, M^+), 254 (3, M^+-29), 228 (12), 184 (35), 157 (34), 134 (34), 110 (17)	36,39–43
$H(C=O)-C(=O)-H$ 60	PFBO-derivative	448	1992	448 (3, M^+, 181 (100)	
$HO-CH=CH-C(=O)-H$ 61	MSTFA-adduct	343	1361 isomeres 1363	343 (9, M^+), 328 (17, M^+-15), 301 (2), 254 (20, M^+-90), 228 (15), 217 (100, M^+-126), 184 (20), 147 (21)	44–47
$HOOC-(CH_2)_7-C(=O)-H$ 62	MSTFA-adduct	443	1993	428 (1, M^+-15), 317 (6, M^+-126), 301 (24), 228 (100), 184 (29), 117 (8)	17,48

Table 2
Data of all detected LPO-products of linolenic acid

Compound	Derivative	Molecular weight	*RI* value	Key fragments (m/z (%))	Literature
$H_5C_2-CH(OH)-C(=O)-H$ 51	MSTFA-adduct	359	1370	344 (2, M^+-15), 330 (2, α-cleavage), 301 (10), 228 (72), 147 (21), 141 (25, α-cleavage	
	PFBO-derivative	355	1471	355 (1, M^+), 340 (55, M^+-15), 326 (100, α-cleavage), 181 (74), 158 (25, M^+-197), 131 (14, α-cleavage)	
$H_5C_2-CH(OH)-CH=CH-C(=O)-H$ 43	PFBO-derivative	381	1750	366 (1, M^+-15), 352 (40, α-cleavage), 200 (39, M^+-181), 184 (25, M^+-197), 181 (100)	42
$HOOC-(CH_2)_7-CH(OH)-C(=O)-H$ 63	MSTFA-adduct	545	2255	530 (5, M^+-15), 317 (57, α-cleavage), 418 (3), 403 (20), 390 (7), 301 (35), 228 (100), 147 (18), 127 (10)	17
	PFBO-derivative	541	2410	526 (7, M^+-15), 360 (4, M^+-181), 344 (22, M^+-197), 326 (100, α-cleavage), 317 (8, α-cleavage), 181 (97), 147 (7), 117 (10)	
$HOOC-(CH_2)_7-CH(OH)-CH=CH-C(=O)-H$ 64	PFBO-derivative	567	2650 2690 isomeres	567 (2, M^+), 552 (18, M^+-15), 386 (25, M^+-181), 370 (80, M^+-197), 352 (78, α-cleavage), 181 (77), 147 (12), 117 (14)	49
$H_5C_2-CH=CH-CH(OH)-C(=O)-H$ 65	PFBO-derivative	381	1715	381 (3, M^+), 352 (13, M^+-29), 326 (44, α-cleavage), 200 (5, M^+-181), 181 (100)	
$H_{13}C_6-CH(OH)-C(=O)-H$ 66	PFBO-derivative	411	1840	396 (7, M^+-15), 326 (83, α-cleavage), 230 (6, M^+-181), 228 (8, M^+-197), 181 (64)	

Table 3
Data of all detected LPO-products of linolenic acid

Compound	Derivative	Molecular weight	*RI* value	Key fragments (m/z (%))	Literature
 67	PFBO-derivative	439	1997	424 (30, M^- 15), 326 (95, α-cleavage), 258 (3, $M^+ - 181$), 242 (29, $M^+ - 197$), 215 (4, α-cleavage), 181 (24)	
 68*	TMS-derivative	470	2483	455 (2, $M^+ - 15$), 439 (1, $M^+ - 31$), 401 (1, α-cleavage), 380 (3, $M^+ - 90$), 313 (5, α-cleavage), 311 (12, 401 − 90), 259 (100, α-cleavage), 223 (6, 313 − 90), 171 (24, α-cleavage), 147 (8), 129 (15)	
 69*	TMS-derivative	470	2504	455 (1, $M^+ - 15$), 441 (1,α-cleavage), 439 (1, $M^+ - 31$), 351 (2, 441 − 90), 299 (48, α-cleavage), 273 (25, α-cleavage), 183 (18, 273 − 90), 147 (10), 131 (100, α-cleavage), 129 (10)	
 70	TMS-derivative hydrogenation product	4744	2500	459 (1, $M^+ - 15$), 443 (2, $M^+ - 31$) 403 (10, α-cleavage), 317 (27, α-cleavage), 313 (4, 403 − 90), 259 (100, α-cleavage), 227 (10, 317 − 90), 173 (57, α-cleavage), 129 (58)	

* The mass spectra didn't allow to predict precisely the location of double bonds.

Table 4
Data of all detected LPO-products of linolenic acid

Compound	Derivative	Molecular weight	*RI* value	Key fragments (m/z (%))	Literature
H_5C_2-CH(OH)-$(CH_2)_3$-CH(OH)-$(CH_2)_{10}$-COOH 71	TMS-derivative hydrogenation product	474	2574	445 (10, α-cleavage), 355 (6, 445 − 90), 384 (1, M^+ − 90), 301 (67, α-cleavage), 275 (37, α-cleavage), 185 (25, 275 − 90), 131 (30, α-cleavage), 129 (100)	
H_5C_2-CH(OH)-$(CH_2)_6$-CH(OH)-$(CH_2)_7$-COOH 72	TMS-derivative, hydrogenation product	474	2581	459 (3, M^+ − 15), 445 (3, α-cleavage), 355 (10, 445 − 90), 317 (7, α-cleavage, 259 (56, α-cleavage), 227 (21, 317 − 90), 131 (52, α-cleavage), 129 (17)	14,50,51

The formation of 2-hydroxyaldehydes with 8 and 10 C-atoms (66 and 67) during LPO of linolenic acid was not expected. These products are probably formed by allylic oxidation of oleic acid [25], they are only present in trace amounts compared to other hydroxyaldehydes.

oxime TMS-ether derivatives or MSTFA adducts and hydroxy compounds as trimethylsilylated derivatives. The identified compounds are listed in Tables 1–4.

Since some of the compounds were not described previously typical mass spectra of a representative of each compound class are discussed: Fig. 1 represents the EI mass spectrum of the PFB oxime TMS-ether derivative of 2-hydroxybutanal 51. Although the molecular ion (m/z = 355) is only of low abundance, the molecular weight can be deduced by a M-15 ion (m/z = 340). Like all EI mass spectra of pentafluorobenzyloximes [31] the MS of 51 shows a prominant peak at m/z 181 corresponding to the benzyloxim residue (Figure 1) and a peak indicating the loss of the oxime part at M-197 (mass 158). The dominant ion of m/z = 326 is caused by α-cleavage after ionization at the trimethylsilylated oxygen.

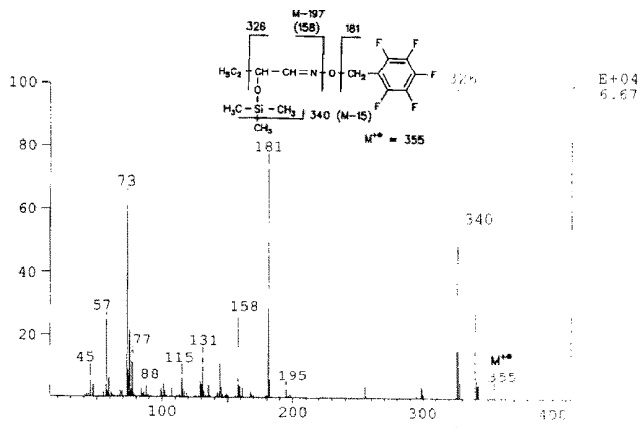
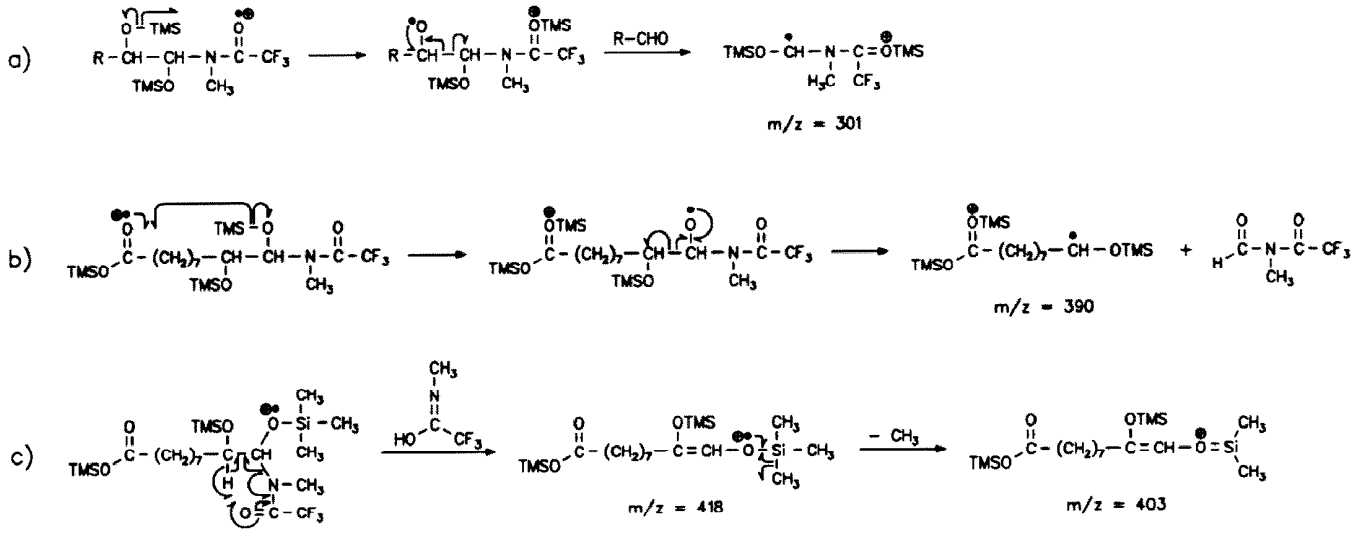


Fig. 1. EI-MS of the O-PFB oxime TMS-ether derivative of 2-hydroxybutanal 51.

A typical mass spectrum of 9-hydroxy-10-oxodecanoic acid 63 converted to its MSTFA adduct is shown in Fig. 2. Apart from the typical aldehyde MSTFA adduct ions at masses 110, 134, 184, 228 [32], the spectrum is characterized by key fragments of mass 317 (resulting from α-cleavage at the trimethylsilylated α-OH group)



Scheme 7: Fragmentation schemes for the production of the ion (a) of the mass 301, (b) of the mass 390, (c) of the mass 403.

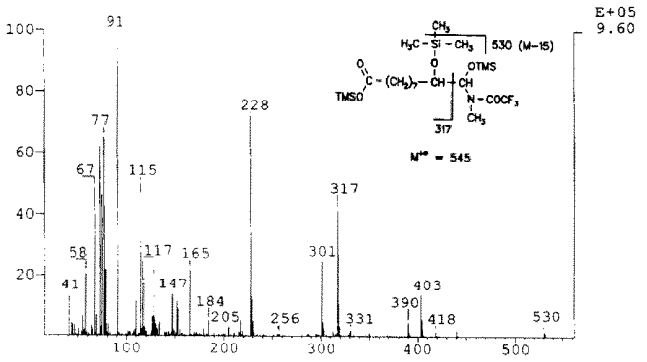


Fig. 2. EI-MS of the MSTFA-derivative of 9-hydroxy-10-oxodecanoic acid 63.

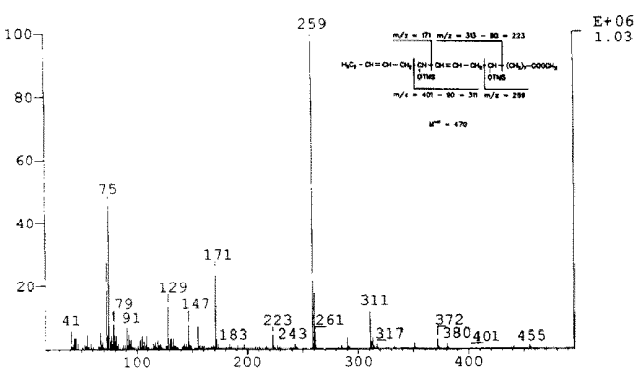


Fig. 3. EI-MS of the bistrimethylsilyl derivative of 9,13-dihydroxyoctadecadienoic acid methyl ester 68.

and one of mass 530 (M-15). Trimethylsilylated ω-oxo-ω-1-hydroxy fatty acids, as 63 tend to suffer after ionization a migration of a TMS group followed by cleavage, thus ions of masses 301 [23], 390 and 403 are produced (Scheme 7). Since 63 carries three TMS groups the spectrum is also characterized by a fragment ion of mass 147, typical for di- and poly-oxygen-trimethylsilylated compounds [52].

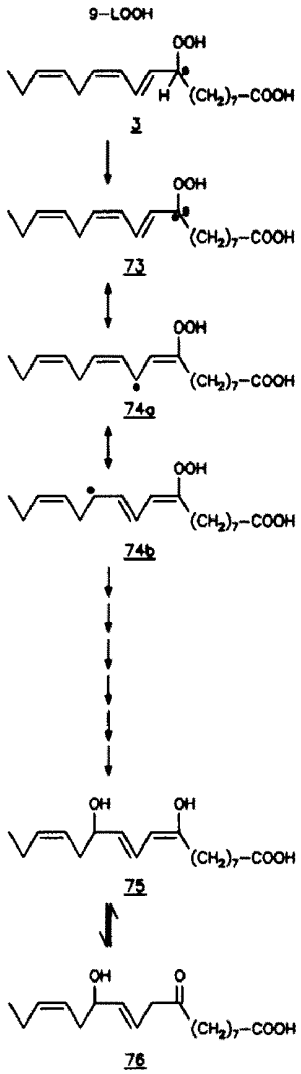
Among the unsaturated dihydroxy fatty acids 9,13- and 12,16-dihydroxyoctadecadienoic acids were detected as methylsilylated derivatives 68 and 69 after reduction with $NaBH_4$.

According to Scheme 3, we expected to find also hydroxy-oxo-octadecadienoic acids, e.g., 31. Although we were able to detect in the GC runs oxo acid derivatives by peaks at the mass 181, (typical for pentafluorobenzyloximes) as well as at mass M-15 and masses due to α-cleavage, we were not able to deduce their structures due to the lack of characteristic peaks indicating the position of the oxo-function.

Therefore we reduced the autoxidation mixture with sodium borohydride after extraction with chloroform and methylation of the acid function. After TLC separation, trimethylsilylation and separation with GC mass spectra were obtained. One of these spectra is reproduced in Fig. 3.

The mass of the molecular ion is deduced by the M-15 fragment at mass 455, one at mass 439 (M-31, loss of $\cdot OCH_3$) and one at mass 380 (M-90, loss of trimethylsilanol). The location of the OTMS groups is unambigiously derived from the key ions of mass 259 and 171 resp. 401 resulting by α-cleavage (see Fig. 3). The fragment of mass 171 also allows to locate one double bond in this fragment, the second double bond can only be localized between carbon 9 and 13. Since this spectrum demonstrates without doubt the oxygen functions in position 9 and 13, the distance of the oxygen function in 1.5 position indicates the predicted reaction sequence 73 → 74a → 74b → 75 → 76 (Scheme 8). The oxo-compound 76 was finally reduced by $NaBH_4$ reduction to 68.

If the double bonds of the fraction with unsaturated dihydroxy fatty acids generated after $NaBH_4$ reduction were reduced with Rh/H_2, 9,13-, 12,16- and 9,16-dihy-



Scheme 8.

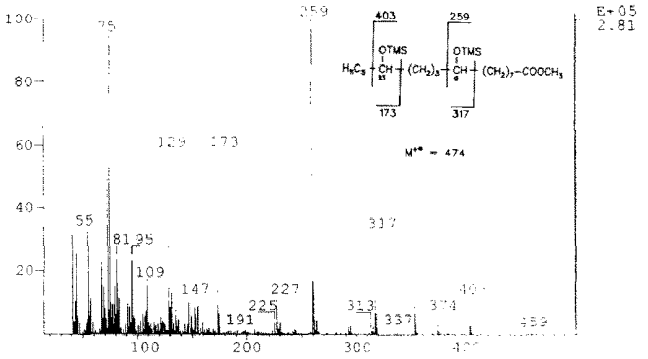


Fig. 4. EI-MS of the bistrimethylsilyl derivative of 9,13-dihydroxyoctadecanoic acid methyl ester 70.

droxy fatty acids (70, 71, 72) were detected. Their mass spectra are very characteristic as exemplified by the spectrum of 70 (Fig. 4). α-Cleavage reactions indicate the location of the oxygen functions.

An oxidation product of linolenic acid is glyoxal 60. As already pointed out 1,2-dioxygenated products are assumed to cause the production of glyoxal, found in tiny amounts also in lipid peroxidation experiments with linoleic acid [25,53]. Glyoxal is obtained probably by abstraction of a hydrogen from activated C-H bond in an already produced unsaturated aldehyde, e.g., 11-oxo-9-undecenoic acid 35 (Scheme 9).

Malondialdehyde 61 was detected in considerable amounts in agreement with earlier investigations [42, 44,45,47] as trimethylsilylated MSTFA adduct in its enolic form as β-hydroxyacrolein.

Discussion

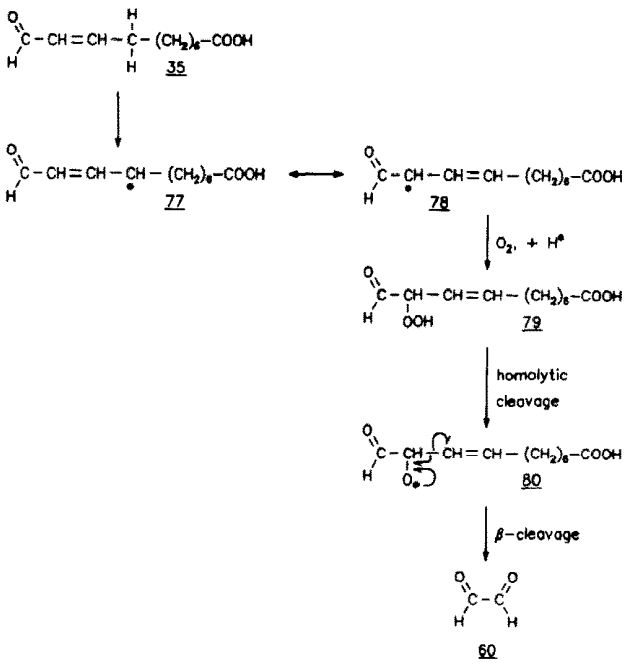
According to Tables 1–4 mainly hydroxy aldehydes (43, 51, 65), their counterparts ω-oxo(ω-1)hydroxy-fatty acids (63) and other oxo-hydroxy fatty acid (64) with none or only one double bond were found after lipid peroxidation of linolenic acid.

In contrast, hydroxy aldehydes (e.g., 37), their counterparts oxo-hydroxy fatty acids (e.g., 41, 49, 57) and other unsaturated aldehydes (e.g., 7, 45, 53) with more than one double bond – also predicted to be produced in LPO of linolenic acid – are missed. This may be explained by the fact that an increasing number of double bonds cause instability [54,55].

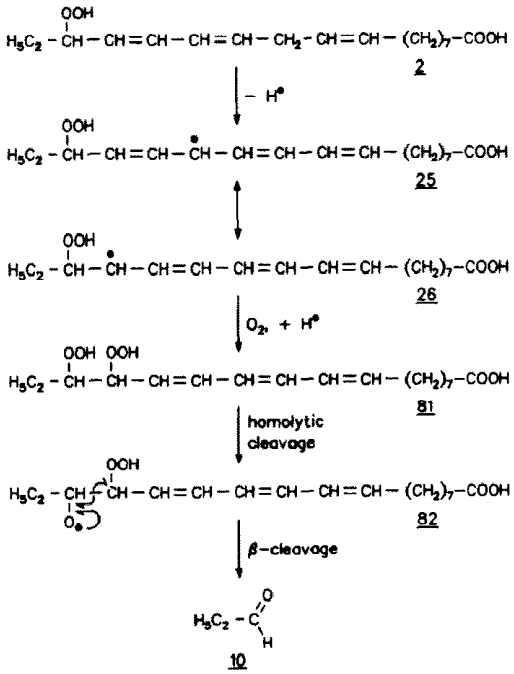
In agreement with the missing of aldehydes with high unsaturation, we were also not able to detect dihydroxy acids with three double bonds (e.g., 27, 28, 29, 30), while dihydroxy acids with two double bonds (68, 69) were present in the mixture of oxidation products.

The occurrence of 1,5-dioxygenated products deserves interest since those products were not detected before in LPO of linolenic acid. Their presence is a strong hint for the original presence of a hydroxy oxo acid: The formation of these oxidation products is visualized via abstraction of a hydrogen from a C-H bond adjacent to the oxygen radical 21 activated as well by a double bond and the oxygen atom, e.g., in 22, resulting finally in an enolic compound which is transformed to the corresponding ketone (see reaction sequence 21 → 23 → 24 → 30 → 31, Scheme 3).

According to Scheme 3 also the production of 1,2-, 1,4-, 1,6-dihydroxy compounds was expected. These



Scheme 9.



Scheme 10.

would also contain three double bonds and consequently are also prone to further reactions.

The occurrence of dioxygenated compounds as lipid peroxidation products of linolenic acid raises another question: The formation of propanal, a main oxidation product of the lipid peroxidation of linolenic acid was always assumed to be caused by decomposition of the radical 6. In the course of this cleavage also a vinyl radical is produced, e.g., 25. Nevertheless, it seems possible that at least a part of the aldehydic compounds may originate from dioxygenated precursors, e.g., 25 may react in the mesomeric form 26 to the dihydroperoxy compound 81. The decomposition of the radical 82 as indicated in Scheme 10 could explain the great amount of propanal found in the lipid peroxidation process of 1. In this case the postulation of the vinylic radical can be avoided and instead an energetical favoured allyl radical would be expelled. The absence of the hydrocarbon 12 may be regarded as indication that vinylic radicals may only be produced to a limited extend. It must be also considered – in respect of recently findings of Boland [56] – that rearrangement reactions of the radical occur, producing a bouquet of unsaturated hydrocarbons.

Probably the oxidation reactions do not stop at the step of dioxygenated products, since the dioxygenated compounds still contain activated allylic C-H bonds. The missing of the predicted compounds with a conjugated system – they contain especially highly activated C-H bonds – may also be an indication for this assumption.

Consequently, trioxygenated products must also be produced as well as compounds representing polyenes. These might be the precursors of lipofuscin [57].

If tissue (e.g., of liver) is worked up, lipid peroxidation products of linolenic and linoleic acid are detected [26,58–61]. As a result, it must be assumed that the here described LPO products will also be found in products of natural origin.

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