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Genesis and metabolisation of 1-pentene during lipid peroxidation of ω-6 unsaturated fatty acids: metabolism of 1-pentene

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Abstract

1-Pentene is generated by decomposition of ω-6 unsaturated fatty acid hydroperoxides. It is transformed under physiological conditions in presence of plant or mammalian liver enzymes to 1-pentene epoxide which can be trapped by reaction with thiophenol. The reaction products were identified by comparing their mass spectra with those of authentic material.

Keywords: 1-Pentene; 1-Pentene epoxide; Lipid peroxidation; 1-Mercaptophenyl-2-pentanol; 2-Mercaptophenyl-1-pentanol; Linoleic acid

1. Introduction

16-Hydroperoxy-9,12-*cis*-14-*trans*-octadecatrienoic acid **1**, a lipid peroxidation product of linolenic acid, reacts in the presence of iron ions by cleavage of the oxygen-oxygen bond producing an alkoxy radical **2** which was shown to decompose to a large number of compounds [1]. Loss of an aldehydic acid leads to an ethyl radical **3** which forms ethylene and ethane by disproportionation. Besides the disproportionation, the ethyl radical can either catch a hydrogen atom from an activated allylic CH_2-group of PUFA or lose a hydrogen atom by reaction with other free radicals, e.g. an alkoxy radical of PUFA. Measurement of ethylene and ethane is used to determine non-invasively the extent of lipid peroxidation [2].

Likewise, decomposition of 13-hydroperoxy-9-*cis*-11-*trans*-octadecadienoic acid **6**, derived during lipid peroxidation from linoleic acid, should provide, via the alkoxy radical **7**, a pentyl radical **8**. This should decompose in analogy to the above deductions to 1-pentene **9** and pentane **10**, but only the occurrence of the latter has been reported [3] (Scheme 1).

Abbreviations: PUFA, polyunsaturated fatty acid; LPO, lipid peroxidation; GC/MS, gas chromatography/mass spectrometry coupling; EO, ethylene epoxide; TRK, Technische Richtkonzentration; TLC, thin layer chromatography; MSTFA, *N*-methyltrimethylsilyltrifluoroacetamide; DMDO, dimethyldioxirane.

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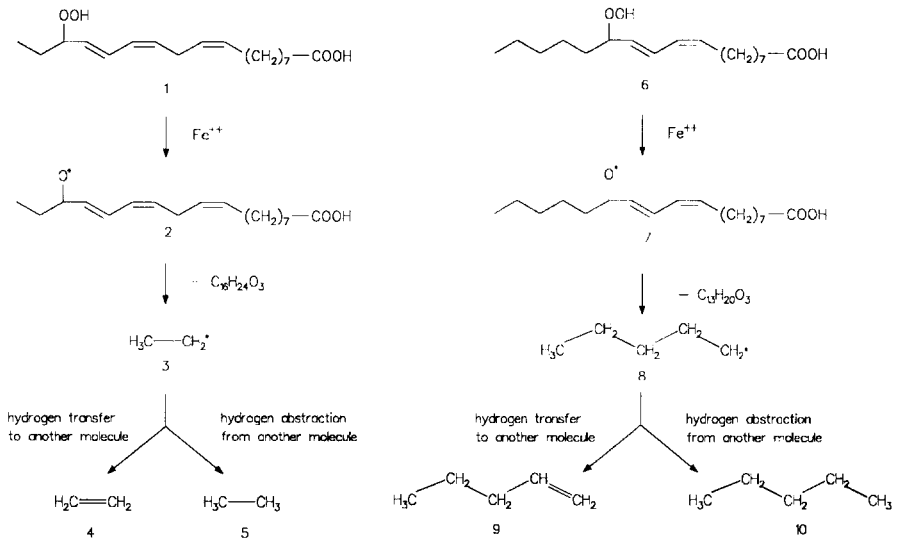


Scheme 1. Genesis of small gaseous hydrocarbons from PUFA during LPO.

Song et al. [4] described an increase in ethylene production by adding linoleic acid to chloroplasts isolated from wheat seedlings. Thus, they concluded that linoleic acid is the precursor of ethylene, while according to the above cited deductions the precursor of ethylene should be a ω-3 PUFA.

Ethylene is metabolized in *Vicia faba* to ethylene epoxide (EO) [6]. Rat liver microsomes were found to convert ethylene to EO [7]. Rats exposed to ethylene exhaled EO [8]. In the hemoglobin and DNA of mice exposed to ethylene, 2-hydroxyethyl-adducts, which are formed by in vivo reaction of EO with lysine, valine, histidine and guanine moieties, were detected [9]. The same kind of metabolism for ethylene was established in man [10]. Like ethylene, propene was shown to be metabolized in vivo to propene epoxide [11]. Therefore, we expected that 1-pentene should be transformed in plant and mammalian tissue also to its epoxide.

Gaseous hydrocarbons are usually analyzed by GC. This method may lead to ambiguous results as recently demonstrated by Kohlmueller et al. [5]: They showed by GC/MS that the main part of the 'pentane peak' corresponds in fact to isoprene and only a small part is pentane. Consequently, we used GC/MS for identification of the gaseous compounds applying headspace analysis.

EO is a carcinogen [12] and mutagen [13]. The exposure to EO in factory work must be limited (TRK 1.000 ml/m^3) [14]. Like propene epoxide and butylene epoxide it causes polyneuropathy [15]. Similar effects should be observed with pentene epoxide.

In this paper we show that 1-pentene is a lipid peroxidation product of ω-6 PUFA. We report further on the in vitro epoxidation of 1-pentene to pentene epoxide **11** using strawberry resp. pig liver homogenates. Although hydroperoxides of fatty acids were shown to epoxidize double bonds in terpenes [16] and plasmalogens [17], they failed to epoxidize 1-pentene in our experiments. The possibility of tissue damage during LPO due to the high reactivity towards nucleophils of pentene epoxide is discussed.

2. Materials and methods

2.1. GC of gas samples

Head space measurements were performed with a fused silica GSQ column (length: 30 m; inner

diameter: 0.53 mm; carrier gas: H_2 2 ml/min; temp. prog.: 60°C isotherm 5 min, 60°–200°C at 10°C/min). Detector: FID; injector temp.: 190°C.; detector temp.: 210°C; split ratio: 1:30; peak area integration: Shimadzu C-R3A Chromatopac.

2.2. GC of trimethylsilylated samples

The same kind of column was used as described for GC/MS. Detector: FID; injector temp.: 270°C; detector temp.: 290°C; split ratio: 1:30; Retention indices were calculated according to Kováts [18] by coinjection of *n*-alkanes.

2.3. GC/MS

GC/MS measurements were carried out using a fused silica DB-1 capillary column (length: 30 m; inner diameter: 0.32 mm; carrier gas: H_2 2 ml/min; temp. prog.: 80°C–280°C at 3°C/min). The GC was coupled to a Finnigan MAT 312 double focussing mass spectrometer operated under EI conditions at 70 eV.

2.4. NMR

NMR measurements were carried out using a Bruker AM 500 NMR spectrometer. Samples were dissolved in deuterated chloroform. Chemical shifts were recorded in ppm.

2.5. GC calibration for gaseous hydrocarbons

A known volume of pure hydrocarbon was diluted with argon in sealed vials. By injection of different amounts of hydrocarbon a calibration diagram showing the relation between quantity and peak area was obtained.

2.6. TLC

TLC was carried out using plates of 20 × 20 cm coated with a 0.75 mm layer of silica gel $60F_{254}$. A 7:3 mixture of cyclohexane and ethylacetate was used as mobile phase. Samples up to 50 mg were separated on one plate.

2.7. Trimethylsilylation

A 0.3-mg aliquot of the substrate was dissolved in 10 μl THF (purified and dried on molecular sieve) and 20 μl MSTFA were added. The mixture was allowed to stand at room temperature for 12 h. Then, 1 μl of the mixture was subjected to GC or GC/MS.

2.8. Headspace analysis of gaseous hydrocarbons

First, 10 mg of linoleic acid resp. linolenic acid were kept under O_2 in a closed 2 ml vial at 37°C for 7 days. Then, 500 μl of the gas phase were injected directly into the GC/MS with a gastight syringe.**4**: R_t = 2 min 42 s; **5**: R_t = 3 min 21 s; **9**: R_t = 18 min 15 s; **10**: R_t = 18 min 37 s.

2.9. Quantification of 1-pentene **9**

First, 1 μmol of linoleic acid was dissolved in 1 ml Borax buffer pH 9.0. Then, 10 μl 0.8 mM $FeSO_4$ and 10 μl 20 mM Na-ascorbate were added to one part of the samples. To another part, 10 μl 1 mM lipoxigenase solution was added. Aliquots (1 ml) of each sample were filled under O_2-atmosphere into 2 ml reactivials, sealed with a rubber stopper and kept at 37°C for 48 h. Then, 100 μl of the headspace atmosphere were injected directly into the gas chromatograph to analyse the gaseous compounds. The amount of 1-pentene and pentane was determined by peak integration and calculated using a calibration curve.

2.10. Epoxidation of 1-pentene **9** with strawberry resp. porcine liver homogenate

First, 40 g of strawberries (purchased at the local market) or 40 g of porcine liver (purchased at a local butchery) were homogenized in a Waring blender containing 40 ml phosphate buffer (pH 7.4). Each homogenate was mixed with 1 ml (9.1×10^{-3} mol) 1-pentene in a closed flask. After 5 days, 1 ml (10×10^{-3} mol) thiophenol was added. The samples were extracted after 1 h with diethylether and subjected to TLC after evaporation of the solvent at 30°C. The fraction

Table 1
Genesis of 1-pentene and pentane from linoleic acid by autoxidation, with Fe^{++}/ascorbate, and with soybean lipoxygenase (reaction time: 48 h; mean values of three independent experiments)

	Autoxidation	Fe^{++}/Na-ascorbate	Lipoxigenase
Yield of 1-pentene [μmol]	$1.45 \times 10^{-4} \pm 1.4 \times 10^{-5}$	$1.52 \times 10^{-2} \pm 1.6 \times 10^{-3}$	$7.95 \times 10^{-2} \pm 5.1 \times 10^{-3}$
Yield of pentane [μmol]	$4.86 \times 10^{-4} \pm 4.9 \times 10^{-5}$	$5.25 \times 10^{-2} \pm 5.7 \times 10^{-3}$	$11.70 \times 10^{-2} \pm 7.6 \times 10^{-3}$
1-pentene:pentane ratio	1:3.35	1:3.45	1:1.47
% of linoleic acid	0.06	7.98	19.66

between R_f = 0.42–0.46 was collected, eluted with diethylether and after evaporation and trimethylsilylation subjected to GC/MS. The compounds 1-mercaptophenyl-2-pentanol and 2-mercaptophenyl-1-pentanol were identified by comparing their mass spectra with those of authentic material.

2.11. Synthesis of 1-pentene epoxide **11**

A 200-μl aliquot (1.83×10^{-3} mol) of pentene was dissolved in 3 ml of CH_2Cl_2 (dried over Alox N and molecular sieve) containing 20 ml dimethyl-dioxirane (0.039 M acetone solution) [19]. The solution was incubated at room temperature for 2 h (pentene epoxide solution).

2.12. Synthesis of 2-mercaptophenyl-1-pentanol **13** *and 1-mercaptophenyl-2-pentanol* **14**

First, 20 ml phosphate buffer (pH 7.4) and 200 μl (1.96×10^{-3} mol) thiophenol were added to the pentene epoxide solution and incubated at room temperature for 12 h. Then the mixture was extracted with diethylether. The residue was subjected to TLC and the fraction between R = 0.44–0.46 was collected and eluted with diethylether. The sample was subjected to GC and GC/MS after evaporation of the solvent and trimethylsilylation. Compounds **13** and **14** were obtained in a 97.3% to 2.7% ratio. Yield: 21.75 mg (**13**);

GC [RI (**13**) = 1640]: 145(100%), 73(53%), 268(12%), 253(8%), 110(6%), 109(5%), 123(5%), 179(3%), 225(2%), 239(1%).

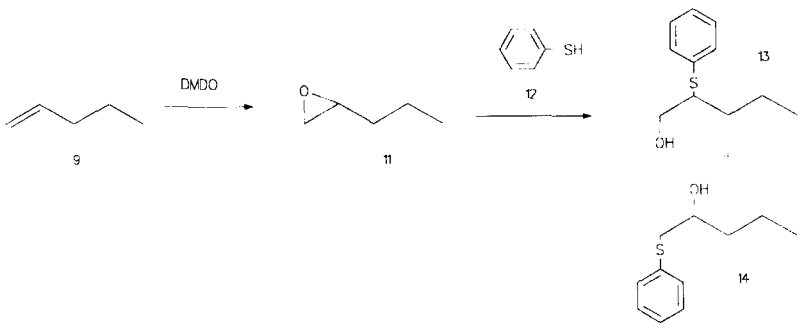
GC [RI (**14**) = 1620]: 165(100%), 123(96%), 268(88%), 73(85%), 167(33%), 110(29%), 109(20%), 159(19%), 253(15%), 178(4%).

^{1}H-NMR (**13**): δ = 7.37 (2H, d, C-2′, J = 7.09 Hz), 7.27 (2H, t, C-3′, J = 7.36 Hz), 7,19 (1H, t, C-4′, J = 7.38 Hz), 3,68 (1H, m, C-2), 3.13 (1H, dd, C-1, H_B, J_1 = 13.65 Hz gem., J_2 = 3.45 Hz *cis*), 2.83 (1H, dd, C-1, H_A, J_1 = 13.65 Hz gem., J_2 = 8.74 Hz *trans*), 2.39 (1H, br, S-OH), 1.55–1.65 (1H, m, H'_A, C-3), 1.40–1.55 (2H, H'_B, C-3 and H''_A C-4), 1.30–1.40 (1H, m, H''_B, C-4), 0.89 (3H, t, C-5, J = 4.59 Hz).

^{13}C-NMR (**14**): δ = 135.30 (C-1′), 129.97 (C-3′), 129.03 (C-2′), 126.54 (C-4′), 69.06 (C-2), 42.20 (C-1), 38.20 (C-3), 18.88 (C-4), 14.01 (C-5).

3. Results and discussion

The autoxidation of linolenic acid resulted in the formation of ethane and ethylene in a 6.53/1 ratio presumably via 16-hydroperoxy-9,12-*cis*-14-*trans*-octadecatrienoic acid. Under the same conditions linoleic acid produces pentane and 1-pentene, but no ethylene or ethane. These findings support the mechanism of the genesis of gaseous hydrocarbons from PUFA as outlined above (Scheme 1). Adding Fe^{++} ions and ascorbic acid to the solution, a system known to promote lipid peroxidation [20], the yield of pentane and 1-pentene was increased up to 7.98% of the linoleic acid, whereas addition of soybean lipoxigenase raised the amount of pentane and 1-pentene up to 19.65×10^{-2} μmol corresponding to 19.66% yield in respect of the starting molecule (Table 1). Products were identified by comparison of their GC retention indices and mass spectra with those of authentic material. Quantification was done by peak area comparison using a GC calibration curve.



Scheme 2. Synthesis of 2-mercaptophenyl-1-pentanol **13** and 1-mercaptophenyl-2-pentanol **14**.

These experiments demonstrate that as expected 13-LOOH derived from linoleic acid produces besides pentane also 1-pentene in analogy to the generation of ethane and ethene by ω-3 hydroperoxides derived from ω-3 PUFA. The fact that 1-pentene had not been observed previously besides pentane may be due to the similarity of their GC retention indices.

The findings of Song et al. [4] noticing an increase of ethylene production after addition of linoleic acid to chloroplasts may be explained by assuming that the ethylene could have derived from hydroperoxidation of linolenic acid contained in the membranes of the chloroplasts.

Determination of 1-pentene should therefore be suitable for estimation of the degree of LPO like it is already commonly done with ethane and pentane [3]. Since in our experiments the distribution pattern of 1-pentene to pentane in cases of autoxidation of linoleic acid (1:3.35 resp. 1:3.45) was found to be different from that obtained in an enzymatic oxidation (1:1.47) this might be a possibility to distinguish between autoxidation or enzymatic oxidation in tissue by determination of the 1-pentene/pentane ratio.

The assumption that 1-pentene might be transformed to its epoxide like ethylene [21], propene [11] and 1,3-butadiene [21] in biological surroundings was checked by adding 1-pentene to strawberry resp. porcine liver homogenates. In these solutions, 1-pentene was oxidized to its epoxide which was trapped by binding to thiophenol **12**: 2-mercaptophenyl-1-pentanol **13** and 1-mercaptophenyl-2-pentanol **14** were obtained indicating that thiophenol is able to open the epoxide at either side although addition at the end methylene group is much preferred. Compounds **13** and **14** were identified by comparison of GC retention indices and mass spectra with those of authentic samples synthesized in our laboratory according to Scheme 2.

Control experiments without addition of 1-pentene did not result in the formation of **13** or **14**. Although this in vitro experiment may only give a hint on the biological pathway in intact tissue, it seems likely that 1-pentene is epoxidized in vivo in a similar way as shown for ethylene, propene or 1,3-butadiene.

Recently we recognized that the double bonds of terpenes [16] and plasmalogens [17] are epoxidized with 13-hydroperoxy linoleic acid. That is why we suspected that a similar reaction could be responsible for the in vivo epoxidation of 1-pentene. However, 13-LOOH added to a solution of 1-pentene did not epoxidize the double bond of the substance, because after addition of thiophenol no 2-mercaptophenyl-1-pentanol **13** or 1-mercaptophenyl-2-pentanol **14** could be detected by GC/MS. These findings suggest that fatty acid hydroperoxides are not the in vivo epoxidizing substance for small hydrocarbons and support the currently accepted theory that a cytochrome P_{450} mono-oxygenase is responsible for this epoxidation [22].

4. Conclusions

Our results point to a previously unknown affect on tissue due to lipid peroxidation of ω-6 PUFA: the formation of a ω-6 hydroperoxy fatty acid causes the release of 1-pentene. This seems especially important with regard to the large portion of ω-6 PUFA contained in lipids. Our experiments strongly suggest that 1-pentene can be metabolised in vivo to 1-pentene epoxide, which presumably acts like ethylene epoxide due to its high reactivity towards nucleophiles. It probably damages DNA and amino acid residues of proteins by alkylating them like ethylene epoxide [9] and propene epoxide [11]. Therefore, the influence of the reaction of 1-pentene and 1-pentene epoxide on body compounds deserves careful study in the future.

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References

[1] W.D. Lehmann, K. Metzger, M. Stephan, U. Wittig, I. Zalan and A.J.R. Habenicht (1995) Anal. Biochem. 224(1), 227–234.
[2] E.N. Frankel, W.E. Neff and E. Selke (1981) Lipids 16(5), 279–285.
[3] L. Massias, E. Postaire, C. Regnault and G. Hazebroucq (1993) Biomed. Chromatogr. 7(4), 200–203.
[4] C. Song, H. Mei, Z. Chu and Y. Cheng (1992) Zhiwu Shengli Xuebao 18(1), 55–62.
[5] D. Kohlmueller and W. Kochen (1993) Anal. Biochem. 210(2), 268–276.
[6] F.B. Abeles and L.J. Dunn (1985) J. Plant Growth Regul. 4, 123–128.
[7] G. Schmiedel, J.G. Filser and H.M. Bolt (1983) Toxicol. Lett. 19, 293–297.
[8] J.G. Filser and H.M. Bolt (1983) Mutat. Res. 120(1), 57–60.
[9] D. Segerbäck (1983) Chem.-Biol. Interact. 45, 139–151.
[10] M. Törnqvist, J. Almberg, S. Nilsson and S. Osterman-Golkar (1989) Scand. J. Work Environ. Health 15, 436–438.
[11] K. Svensson, K. Olofson and S. Osterman-Golkar (1991) Chem.-Biol. Interact. 78, 55–66.
[12] R.P. Beliles and J.C. Parker (1989) Health Phys. 57, 333–340.
[13] A. Basler (1985) BGA Schr., (4, Gesund.Bewertung Ausgewählter Chem. Stoffe), pp. 43–50.
[14] Praxissoftware Gefahrstoffe Version 1.0 (1995) WEKA Fachverlag für technische Führungskräfte.
[15] A. Ohnishi and Y. Murai (1993) Environ. Res. 60(2), 242–247.
[16] W. Meyer and G. Spiteller (1993) Liebigs Ann. Chem. 1993, 17–19.
[17] C. Scheick and G. Spiteller (1993) Liebigs Ann. Chem. 1993, 1245–1248.
[18] E. Kováts (1958) Helv. Chim. Acta 41, 1915.
[19] W. Adam, L. Hadjiarapoglou and A. Smerz (1991) Chem. Ber. 124, 227–232.
[20] A. Loidl-Stahlhofen, W. Kern and G. Spiteller (1995) J. Chromatogr. (B) Biomed. Appl. (in press).
[21] J.G. Filser and H.M. Bolt (1984) Arch. Toxicol. 55, 219–223.
[22] H.M. Bolt, J.G. Filser and F. Strömer (1984) Arch. Toxicol. 55, 213–218.