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2-Hydroxy-Succinaldehyde, a Lipid Peroxidation Product Proving that Polyunsaturated Fatty Acids are Able to React with Three Molecules of Oxygen

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2-Hydroxy-succinaldehyde was detected by a GC/MS analysis of trapped aldehydic compounds obtained after Fe^{2+}/ascorbate lipid peroxidation of arachidonic acid. Precursor molecules of aldehydes are hydroperoxy compounds. Thus the generation of the two aldehydic groups in 2-hydroxysuccinaldehyde requires a precursor molecule with two hydroperoxy groups. The hydroxy group in 2-position is generated by a third hydroperoxidation reaction. The detection of 2-hydroxysuccinaldehyde—although found only in traces—is the first example for triple dioxigenation of unsaturated fatty acid. Linolenic acid produces 2-hydroxysuccinaldehyde in much lower amounts than arachidonic acid. A similar oxidation of linoleic acid was not observed.

Keywords: Lipid peroxidation, 2-hydroxysuccinaldehyde, arachidonic acid, linoleic acid, linolenic acid

Abbreviations: BHT, 2,6-di-tert.-butyl-4-methyl-phenol; GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; LH, unsaturated fatty acids; LOOH, hydroperoxy fatty acids, derived from unsaturated fatty acids; LOH, hydroxy fatty acids, derived from unsaturated acids; LPO, lipid peroxidation; MDA, malon-dialdehyde; MSTFA, N-methyl-N-trimethylsilyl-trifluoroacetamide; PFBA HCl, pentafluorobenzylhydroxylamine; PFBO; pentafluorobenzyl-oxime; TMS; trimethylsilyl; PUFA's, polyunsaturated fatty acid(s).

INTRODUCTION

Aldehydic compounds, which are produced by Fe^{2+} initiated cleavage of lipid hydroperoxides of polyunsaturated fatty acids (PUFAs), can be trapped by immediate addition of pentafluorobenzylhydroxylamine (PFBHA) to the oxidation mixture. PFBHA reacts fast with carbonyl groups.[1–3] The resulting pentafluorobenzyloximes (PFBO) derivatives were reacted with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) in order to protect OH groups and enhance the volatility necessary for GC separation. The GC effluents were introduced in a mass spectrometer. PFBO derivatives of aldehydes are characterized in their mass spectra by a typical key ion of mass 181, indicating the pentafluorobenzyl residue. If the ion current of this ion is registered, only products are measured which contain a pentafluorobenzyl group. Compared to conventional mass spectrometry the sensitivity is increased

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by a factor of approximately 100 and more—therefore even traces of aldehydes are indicated by this method.[3]

In the course of the investigation of oxidation products of linoleic acid[3] we detected that some aldehydic products showed in their mass spectrum a typical second key ion: It had the mass 326 and turned out to be characteristic for trimethylated PFBO derivatives of 2-hydroxyaldehydes which is formed in a typical α-cleavage reaction.

Compounds of this type are also produced from linolenic acid[4] and arachidonic acid.

We describe in this paper the application of the above outlined method to trap 2-hydroxysuccinaldehyde, a previously unknown oxidation product of arachidonic acid.

MATERIAL AND METHODS

Materials

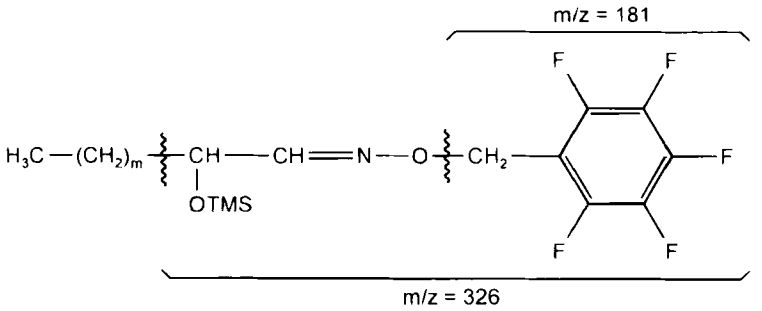
N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Macherey & Nagel (Düren, Germany). All other chemicals were purchased from Fluka (Neu Ulm, Germany). Solvents, obtained from Merck (Darmstadt, Germany) were destilled before use. The fatty acids were stored at –18°C under argon.

Fe2+/Ascorbate Autoxidation of Fatty Acids

15 mg of fatty acids were incubated in 23 ml of 0,1 M TRIS/HCl-buffer (pH = 7,4) and 45 ml of 0,15 M aqueous KCl solution containing 3,8 ml of 0,8 mM Fe^{2+} sulfate and 3,8 ml of 20 mM ascorbate (sodium salt)[5] for 24 h at room temperature. Oxidation of fatty acids was stopped after 24 h by addition of 80 µl BHT (2% in methanol).

Preparation of PFBO Derivatives of the Oxidation Products

The carbonyl groups of LPO products were transformed to pentafluorobenzyloxime derivatives by the methods described by VanKuijk[1] resp. Hoffmann.[2] Briefly, 23 ml of a solution of



SCHEME 1 PFBO-TMS-derivative of an α-hydroxyaldehyde

0,05M PFBA HCl in methanol was added to the raw methanolic oxidation mixture. The mixture was kept for one hour at room temperature. The PFBO-derivatives were extracted three times with 70 ml $CHCl_3$. The solvent of the organic fraction was evaporated and the residue redissolved in ethyl acetate. An aliquot (0,2 mg) of PFBO-derivatives was added to 10 µl MSTFA for trimethylsilylation. After keeping overnight at room temp. a sample of the trimethylsilylated products was subjected to separation by GC/MS. Besides total ion current measurement also the ion currents of the ions of mass 181 and of mass 326 were registered. Full mass spectra were recorded from the peaks for compound identification.

Detection of the PFB-Oxime TMS-Ether Derivative of 2-Hydroxy Succinaldehyde

GC (DB-1): RI = 2192/2196

MS [relative intensity (%)] = 73 (28), 161 (9), 181 (100), 195 (10), 225 (11), 326 (95), 340 (6), 367 (8), 383 (5), 474 (2), 549 (10), 564 (3).

Gas Chromatography/Mass Spectrometry

GC was carried out with a Carlo Erba HRGC 5160 Mega Series chromatograph equipped with a flame ionisation detector, using a DB-1 fused-silica glass capillary column (30 m × 0.32 mm i.d.), temperature programmed from 80°C to 280°C at 3°C/min. The temperature of the injector and detector were kept at 270°C 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 ionisation 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.

RESULTS

Pure linoleic, linolenic and arachidonic acid were subjected to oxidation with Fe^{2+}/ascorbate according to a procedure described by Esterbauer.[5] After 24 h reaction time BHT was added to stop oxidation. Produced aldehydes were trapped immediately by reaction with pentafluorobenzylhydroxylamine.[1–3] The chloroform extract was analyzed by GC/MS after trimethylsilylation with MSTFA as described earlier using the ion currents of mass 181 and 326 to identify α-hydroxyaldehydes.

Identification of 2-hydroxysuccinaldehyde was achieved from its mass spectrum. The molecular weight was detected at mass 564, confirmed by a M-15 ion (m/z = 549). The ions at mass M-197 (m/z = 367) and one at mass M-181 (m/z = 383) are typical fragments for PBFO derivatives of aldehydes, they indicate loss of the PFB residue.[3]

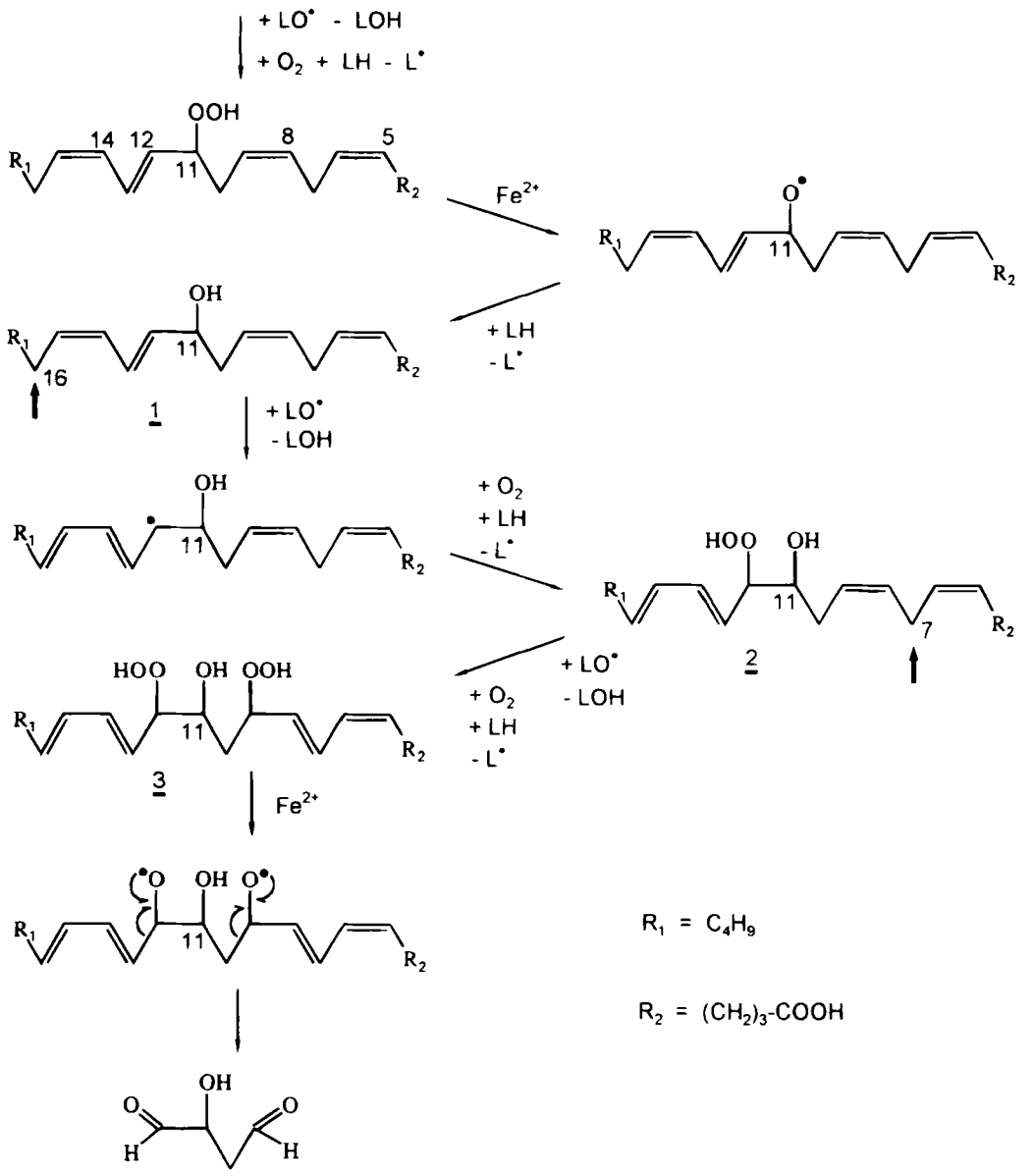
The alternative α-cleavage product of mass 340 was also detected, but only in low yield. The high intensity of the ion of mass 181 is an indication that two pentafluorobenzyl groups were present. Considering the molecular weight and the cleavage products the structure of the compound was deduced to be that of 2-hydroxysuccinaldehyde.

2-Hydroxysuccinaldehyde was detected especially after oxidation of arachidonic acid. It amounts to approximately 1/100 of α-hydroxyheptanal[7] or malondialdehyde.[8] Only just detectable amounts of 2-hydroxysuccinaldehyde were found after oxidation of linolenic acid. It was not detected after oxidation of linoleic acid.

DISCUSSION

The production of 2-hydroxysuccinaldehyde after oxidation of arachidonic acid indicates a subsequent attack of three oxygen molecules on one molecule of arachidonic acid. As is well known, nonenzymic oxidation of arachidonic acid produces six monohydroperoxides.[9,10] Such LOOH molecules are cleaved by Fe^{2+} to LO· radicals, they can pick up a hydrogen atom from another molecule to produce unsaturated hydroxy acids (LOHs). LOHs are also produced enzymically from LOOH in biological media.[11] Recently we demonstrated that these products are subjected to a second oxygen attack by removal of hydrogen from an activated C-H bond adjacent to the diene system, this induces the generation of α-hydroxyaldehydes[6]—they also were detected later in biological samples.[12,13] 2-Hydroxysuccinaldehyde is a α-hydroxyaldehyde. Its genesis follows obviously the outlined pattern in Scheme 2.

2-Hydroxysuccinaldehyde contains an aldehyde group in addition to the α-hydroxy aldehydic group. This requires the presence of an additional

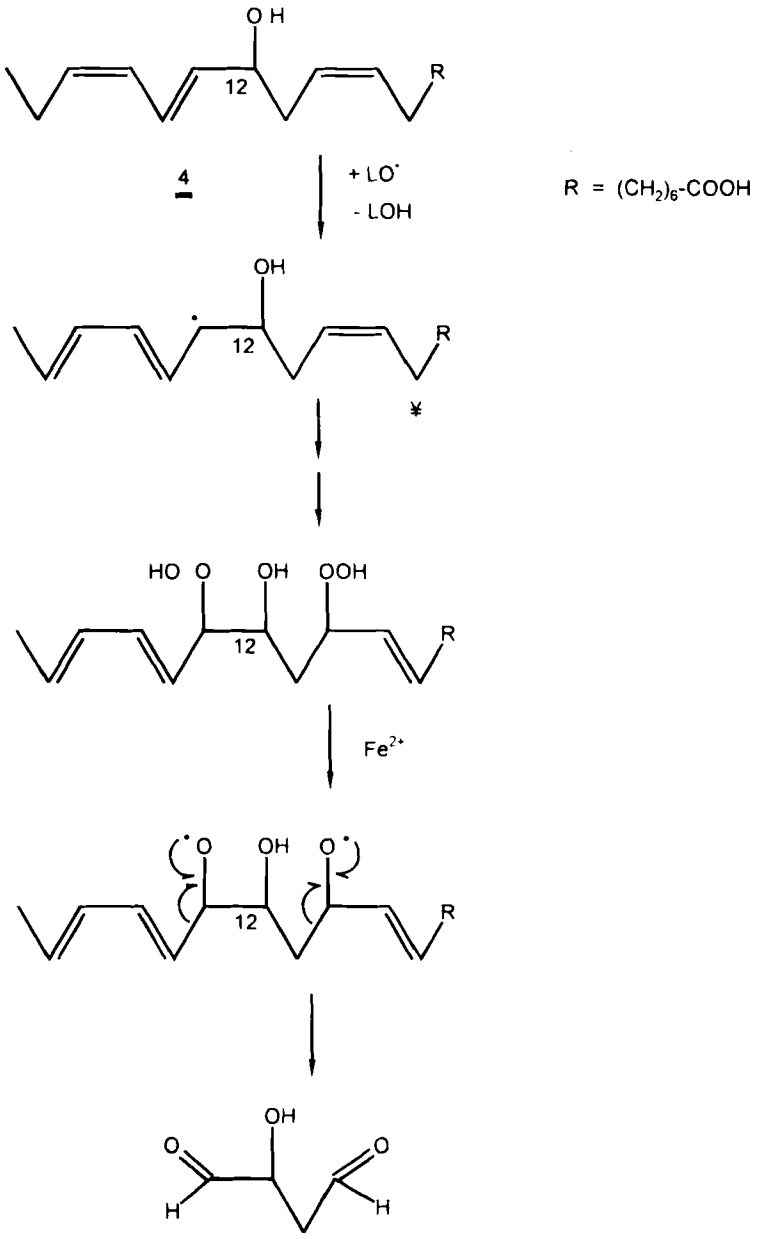


SCHEME 2 Mechanism of the generation of 2-hydroxysuccinaldehyde by triple attack of oxygen to arachidonic acid

hydroperoxyl group in a precursor molecule. Since in 2-hydroxysuccinaldehyde the end groups of arachidonic acid (C_5H_{11} resp. COOH) are missing, it should be produced by oxidation of the double bond system in the middle part of arachidonic acid. Therefore, we speculate that 2-hydroxysuccinaldehyde is either produced from 9-hydroxy-5-cis,7-trans,11-cis, 14-cis-eicosa-tetraenoic acid or, as outlined in scheme 2, 11-hydroxy-5-cis, 8-cis,12-trans, 14-cis-eicosatetranoic acid ***1***. Removal of hydrogen in position 16 of ***1*** would produce 11-hydroxy-12-hydroperoxy-5,8,13,15-eicosat traenoic acid **2**, which still possesses a double allylically activated CH_2 group in position 7. Therefore, an $LO^{\cdot}$ radical may remove a hydrogen radical from position 7 causing the attack of a third

oxygen molecule at position 9 and resulting in production of 11-hydroxy-9,12-dihydroperoxy-5,7,13,15-eicosatetraenoic acid **3**. Fe^{2+}-induced cleavage of the two hydroperoxy groups in position 9 and 12 of **3** would produce 2-hydroxysuccinaldehyde (Scheme 2):

Linolenic acid is also able to produce 2-hydroxysuccinaldehyde, but only in just detectable amounts. In this case the starting molecule is either 12-hydroxy-9,13,15-octadecatrienoic acid **4** (Scheme 3) or 13-hydroxy-9,11,15-octadecatrienoic acid **5**:



SCHEME 3 Mechanism of the generation of 2-hydroxysuccinaldehyde by triple attack of oxygen to linolenic acid

Both molecules (as well as 8- and 11-hydroxides of arachidonic acid) possess only one activated CH_2 group adjacent to the diene system. Consequently after production of an α-hydroxyhydroperoxy compound, the introduction of the second necessary hydroperoxy-group for 2-hydroxysuccinaldehyde is suppressed, due to lack of sufficient activation (only by one double bond) of the CH_2-groups labeled with ¥ in Scheme 3. Thus the yield of 2-hydroxysuccinaldehyde from linolenic acid is much lower than from arachidonic acid.

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