Monatshefte für Chemie 122, 719–724 (1991)

Monatshefte für Chemie
Chemical Monthly
© Springer-Verlag 1991
Printed in Austria

Ozonolysis of Olefins, V [1]: Emulsion Ozonization of Methyl Linoleate and Methyl Linolenate in Aqueous Alkaline Hydrogen Peroxide

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Summary. The ozonolysis of methyl linoleate and methyl linolenate in neutral and alkaline aqueous emulsions of hydrogen peroxide was investigated. Besides the expected products such as dimethyl malonate (**3b**), dimethyl azelate (**3h**) and methyl hexanoate (**2a**) further homologous methyl esters of dicarboxylic acids (**3a–g**), oxo carboxylic acids (**4a, b, e–h**) and hydroxy carboxylic acids (**4c, d**) could be detected by GC/MS analysis. Furthermore a method for separation of 8-hydroxyoctanoic acid (**4d**) by methylation and extraction of the reaction mixture containing the ozonolysis products is described.

Keywords. Ozonization; Fatty esters, 8-Hydroxyoctanoic acid.

Ozonolyse von Olefinen, V [1]: Ozonolyse von Linol- und Linolensäuremethylester in wäßriger alkalischer Emulsion von Wasserstoffperoxid

Zusammenfassung. Die Ozonolyse von Linol- und Linolensäuremethylester wurde in neutraler bzw. alkalischer wäßriger Emulsion von Wasserstoffperoxid untersucht. Dabei wurden im Reaktionsgemisch mittels GC/MS neben Malonsäuredimethylester (**3b**), Azelainsäuredimethylester (**3h**) und Hexansäuremethylester (**2a**) weitere homologe Dicarbonsäuren (**3a–g**), Oxocarbonsäuren (**4a, b, e–h**) und Hydroxycarbonsäuren (**4c, d**) nachgewiesen. Weiters wurde eine Methode ausgearbeitet, mit der es gelang, 8-Hydroxyoctansäure (**4d**) – eine hinsichtlich ihrer biologischen Aktivität bedeutende Verbindung – durch Methylierung und Extraktion aus dem Reaktionsgemisch abzutrennen.

Introduction

Ozonolysis of fatty acid derivatives plays an enormous role for structure analysis [2] as well as for the preparation of technically important intermediates like azelaic acid [3]. Most recently the isolation of alkyl 3,3-dialkoxypropanoates by ozonolysis of polyunsaturated fatty acid esters in a solution of HCl in methanol was reported [4]. Furthermore the mechanism of ozone attack upon lipids has been studied in order to explain the toxic effect of ozone [1]. So it has been found that reaction of ozone with unsaturated fatty acids in aqueous emulsions leads to the formation of lipid peroxidation products [5]. Ozonolysis under oxidative conditions can either be performed by thermal autoxidation [6], treatment with performic acid [7–9] or by reaction in alkaline, aqueous emulsion [10]. Thus, the emulsion ozonization of cycloolefins in hydrogen peroxide led to alkanedicarboxylic acids in one step

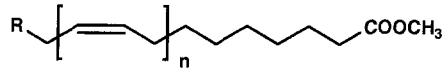
and good yields [10]. In most cases, however, oxidative ozonolysis leads to secondary oxidation products like esters, hydroxy- and formyloxyderivatives and a mixture of homologous dicarboxylic acids [7]. Investigations with methyl oleate have shown that addition of small amounts of water can reduce the yield of side products, further addition, however, leads to an increase of concentration of side products [11, 12].

Because the emulsion ozonization of fatty acids mainly was carried out with oleic acid, in the present study the ozonolysis is extended to methyl linoleate (**1 a**) and linolenate (**1 b**) in order to examine the influence of reaction conditions in the yield of secondary products.

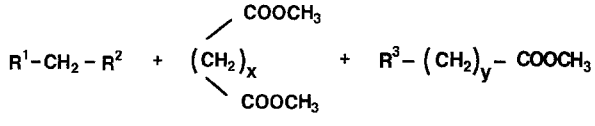
Results and Discussion

Alkaline Aqueous Ozonization

By ozonolysis of methyl linoleate (**1 a**) and methyl linolenate (**1 b**) in aqueous alkaline emulsions of hydrogen peroxide and keeping the reaction mixture at room temperature for two days many byproducts could be obtained. After working up and methylation, besides the expected products such as dimethyl malonate (**3 b**), dimethyl azelate (**3 h**) and methyl hexanoate (**2 a**) further homologous methyl esters



1 a,b



2 a-d **3 a-h** **4 a-h**

1	n	R
a	2	$CH_3(CH_2)_3$
b	3	CH_3

2	R^1	R^2
a	$CH_3(CH_2)_3$	$COOCH_3$
b	$CH_3(CH_2)_3$	CHO
c	$CH_3(CH_2)_2$	CHO
d	$CH_3(CH_2)_3$	OH

3	x
a	0
b	1
c	2
d	3
e	4
f	5
g	6
h	7

4	y	R^3
a	6	CHO
b	7	CHO
c	6	OH
d	7	OH
e	5	CH_3CO
f	6	CH_3CO
g	7	CH_3CO
h	7	O-CHO

of dicarboxylic acids (**3a–g**), oxo carboxylic acids (**4a, b**), (**4e–h**) and hydroxy carboxylic acids (**4c, d**) could be detected. A mechanism for the formation of these side products has been proposed by Pasero et al. [11, 12]. Thus, in the first step a formation of formoxy esters is discussed, either by loss of hydrogen and carbon monoxide from the ozonide or by an anomalous stabilization of the carbonyl oxide ion resulting from the cleavage of an ozonide. Most of the carboxylic acid derivatives detected can be assumed to result from hydrolytic cleavage of such formoxy esters.

Neutral Aqueous Ozonization

Different results were obtained when ozonolysis was conducted in neutral aqueous emulsions followed by oxidative workup in alkaline hydrogen peroxide at room temperature. Surprisingly, in the case of methyl linolenate (**1b**) three main products such as 7-formyl methyl heptanoate (**4b**), 8-hydroxy methyl octanoate (**4d**) and dimethyl nonanedioate (**3h**) were obtained with the following peak area ratio: **4a**:**4d**:**3h** = 1:0.72:0.95. Malonic acid as well as other homologous dicarboxylic acids were found only in low quantities. In the case of methyl linoleate (**1a**) further byproducts such as methyl hexanoate (**2a**), hexanal (**2b**), pentanal (**2c**) as well as pentanol (**2d**) could be detected. These products should result from analogous side reactions of the fragment obtained during ozonolysis containing the alkyl terminal of the fatty ester.

Analogous ozonization and treatment of the ozonized products at elevated temperature in alkaline hydrogen peroxide led again to an increase of byproducts. The only difference to the results obtained at room temperature was the lower quantity of dicarboxylic acids (**3a–h**) as well as the appearance of the oxo carboxylic acids (**4e–g**).

Because treatment of dimethyl nonanedioate and methyl octadecanoate with ozone under the same conditions gave no side reactions, formation of these products requires olefinic double bonds in the starting material. They must have been formed within ozonolysis or following oxidative workup.

Among the three main products resulting from methyl linolenate methyl 8-hydroxyoctanoate (**4d**) is considered to be the most interesting one. This compound as well as its hydroperoxy derivative is also formed in lipid peroxidation [13]. It could be proved that these products inhibit the metabolism of tumour cells, especially glycolysis and respiration.

Although **4d** has been detected in the ozonolysis of fatty esters, it has never been formed in higher yields and was therefore not separated from other byproducts. In order to isolate methyl 8-hydroxyoctanoate, the reaction mixture containing 8-hydroxyoctanoic acid, 7-formyl heptanoic acid and nonanedioic acid was treated with BF_3/methanol at 60 °C. Water was added and dimethyl nonanedioate (**3h**) could be separated by extraction with *n*-hexane. By further extraction of the aqueous layer with dichloromethane methyl 8-hydroxyoctanoate (**4d**) could be obtained in 20% yield. The dimethoxy derivative of methyl 7-formyl heptanoate (**4b**), which should also be obtained under these conditions, could not be found; acid catalyzed side reactions or decomposition may have occurred during workup.

Experimental

Methyl linoleate and linolenate were from Sigma G.m.b.H., FRG; all compounds had a purity over 99% (GC).

A Hewlett Packard instrument MSD (combination of gas chromatograph 5890 and mass spectrometer 5970) was used for separation and detection; fused-silica capillary column, 30 m × 0.32 mm, 0.25 μm DB-5 (J & W Scientific Inc.). The identification of the different compounds was possible either by comparison of the mass spectra with those of authentic samples, or in case of hydroxy acids by comparison with the spectra of *TMS* derivatives.

Ozonolysis in Emulsions of Alkaline Hydrogen Peroxide

70 ml of water containing 900 mg NaOH and 1.2 g H_2O_2 (30%) was added to 250 mg (0.85 mmol) methyl linoleate. A stream of an O_2/O_3 mixture containing 4% ozone was passed through this stirred solution at 0 °C for 0.5 h. The reaction mixture remained at room temperature for two days. Afterwards the solution was acidified with HCl conc. and extracted with diethyl ether for three times. After removal of the solvent the residue was treated with diazomethane in order to get the methyl esters for GC/MS analysis.

Relative peak area ratios: **3a** (6.10%), **3b** (5.80%), **3c** (18.71%), **3d** (12.33%), **3e** (7.29%), **3f** (4.37%), **3g** (9.09%), **3h** (27.05%).

440 mg (1.5 mmol) methyl linolenate was ozonized as described above to give a reaction mixture with the following peak area ratios (MS-detector): **3a** (1.85%), **3b** (2.20%), **3c** (4.51%), **3d** (4.27%), **3e** (3.27%), **3f** (4.97%), **3g** (6.54%), **3h** (37.24%), **4b** (3.34%), **4d** (2.63%), **4e** (0.9%), **4f** (0.75%), **4g** (8.93%).

Methyl 8-Hydroxyoctanoate (**4d**) [14]

m/e = 144 (11), 143 (4.5) $[M\text{-}OCH_3]^+$, 124 (10), 115 (4), 101 (19), 96 (20), 87 (49), 74 (100), 55 (75), 43 (48), 41 (50). The identification was achieved by derivatization of **4d** with BSTFA.

TMS-ether of **4d** [14]: m/e = 231 (22) $[M\text{-}CH]^+$, 199 (82), $[M\text{-}C_2H_7O]^+$, 181 (3), 159 (4), 124 (6), 103 (22) $[CH_2O\mathit{TMS}]^+$, 89 (38), 73 (65) $[\mathit{TMS}]^+$, 55 (100).

Methyl 7-Oxooctanoate (**4e**) [15]

m/e = 141 (3) $[M\text{-}OCH_3]^+$, 140 (4) $[M\text{-}CH_3OH]^+$, 125 (3), 115 (30) $[M\text{-}C_3H_5O]^+$, 114 (6), 98 (4), 95 (14), 87 (12), 83 (10), 69 (15), 55 (23), 43 (100) $[CH_3CO]^+$.

Methyl 8-Oxononanoate (**4f**)

m/e = 155 (2) $[M\text{-}OCH_3]^+$, 154 (3) $[M\text{-}CH_3OH]^+$, 139 (2), 129 (26) $[M\text{-}C_3H_5O]^+$, 109 (9), 97 (27), 87 (18), 69 (27), 55 (26), 43 (100) $[CH_3CO]^+$.

Methyl 9-Oxodecanoate (**4g**)

m/e = 169 (6) $[M\text{-}OCH_3]^+$, 143 (15) $[M\text{-}C_3H_5O]^+$, 111 (26), 97 (7), 83 (27), 69 (14), 55 (36), 43 (100) $[CH_3CO]^+$.

Ozonolysis in Neutral Aqueous Emulsion

70 ml of water was added to 250 mg (0.85 mmol) methyl linoleate. The ice cooled mixture was stirred and an O_2/O_3 mixture containing 4% ozone was passed through it for 0.5 h. Then 900 mg NaOH and 1.2 g H_2O_2 (30%) were added and the reaction mixture was heated to reflux temperature until all peroxidic material had been destroyed. After acidification, extraction with diethyl ether and treatment with diazomethane the mixture was analyzed by GC/MS to give the following peak area ratios: **2a** (0.74%), **3b** (0.58%), **3c** (0.28%), **3d** (0.37%), **3e** (0.92%), **3f** (5.02%), **3g** (14.45%), **3h** (44.04%), **4a** (2.98%), **4c** (2.26%), **4d** (13.21%), **4e** (1.09%), **4f** (1.62%), **4h** (6.22%).

Methyl 7-Formylheptanoate (**4a**) [16]

m/e = 144 (14) [*M*-28], 141 (25) $[M\text{-}OCH_3]^+$, 129 (45), 112 (6), 101 (19), 87 (100), 74 (98), 69 (78), 55 (79), 41 (94), 29 (74).

Methyl 7-Hydroxyheptanoate (**4c**)

m/e = 130 (16), 129 (5) $[M\text{-}OCH_3]^+$, 110 (14), 115 (4), 101 (25), 87 (49), 74 (100), 69 (6), 55 (68), 43 (50), 41 (60), 31 (43). The identification was possible by the *TMS*-derivative.

TMS-ether of **4c**: m/e = 217 (30) $[M\text{-}CH_3]^+$, 185 (100) $[M\text{-}C_2H_7O]^+$, 159 (9), 141 (7), 129 (10), 103 (30) $[CH_2OTMS]^+$, 89 (52), 75 (70), 73 (83) $[TMS]^+$, 55 (86).

Methyl 8-Formoxyoctanoate (**4h**) [17]

m/e = 171 (8) $[M\text{-}OCH_3]^+$, 125 (3) $[M\text{-}OCH_3, HCOOH]^+$, 124 (11) $[M\text{-}C_2H_6O_3]^+$, 96 (16), 87 (21), 74 (100), 55 (64), 41 (40).

300 mg (1.02 mmol) of methyl linolenate were ozonized in the same way to give a reaction mixture with the following peak area ratios: **3b** (0.45%), **3c** (0.35%), **3d** (0.54%), **3e** (1.34%), **3f** (6.10%), **3g** (10.47%), **3h** (38.55%), **4c** (5.43%), **4d** (28.17%), **4e** (1.29%), **4f** (1.32%).

Methyl 8-Formyloctanoate (**3b**) [16]

m/e = 158 (10), 155 (18) $[M\text{-}OCH_3]^+$, 143 (30), 126 (3), 115 (12), 111 (37), 87 (72), 74 (100), 55 (97), 41 (80), 29 (70).

An ice cooled emulsion of 230 mg (0.78 mmol) of methyl linoleate in 70 ml of water was stirred and an O_2/O_3 mixture containing 4% ozone was passed through it for half an hour. Then 900 mg NaOH and 1.2 g H_2O_2 was added and the reaction mixture was kept at room temperature for 2 days. Further steps were similar as described above.

Peak area ratios: **2a** (6.18%), **2b** (1.78%), **2c** (1.98%), **2d** (1.19%), **3g** (2.88%), **3h** (30.75%), **4a** (22.02%), **4b** (3.71%), **4d** (12.49%).

Similarily 200 mg (0.68 mmol) of methyl linolenate was ozonized to give the following peak area ratios: **3g** (3.9%), **3h** (27.0%), **4a** (28.5%), **4b** (7.0%), **4d** (20.5%), **4h** (1.29%).

Separation of Methyl 8-Hydroxyoctanoate (**4d**)

Ozone was passed through a stirred ice cooled emulsion of 680 mg (2.32 mmol) methyl linolenate in 70 ml of water for 0.5 h. Then 1.0 g NaOH and 3.0 g H_2O_2 (30%) were added and the reaction mixture was kept at room temperature for two days. The mixture was acidified with HCl conc. and extracted with 50 ml of diethyl ether. The organic layers were collected, dried and the solvent evaporated. Then 5 ml of a BF_3/methanol solution (50%) were added to the residue and the mixture heated at a temperature of 60–65°C for 15 min.

The mixture was extracted two times with 20 ml of *n*-hexane, thus separating **3g** and **3h**, whereas most of **4d** remained in the aqueous layer. GC analysis of the organic layer provided the peak area ratios **3g**:**3h**:**4d** = 0.088 : 1 : 0.85. In order to separate **4d**, the aqueous layer was extracted twice with dichloromethane. After removal of the solvent the oily residue was distilled under reduced pressure to give 80 mg of **4d** (20% yield).

The product was identified by GC/MS by comparison with an authentic sample.

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Received November 22, 1990. Accepted December 5, 1990