

^1H -NMR Spectroscopy Study of Oleic Acid and Methyl Oleate Ozonation in different reaction conditions

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Palabras clave: Ozono, resonancia magnética nuclear, ácido oleico, oleato de metilo, ozonización, reactor burbujeante.

Key words: Ozone, ^1H -NMR, Oleic acid, Methyl oleate, Ozonation, Bubbling reactor.

RESUMEN: Las reacciones de ozono con ácido oleico u oleato de metilo se llevaron a cabo en presencia de hexano, tetracloruro de carbono, etanol o agua. Todas las mezclas de reacción fueron estudiadas a través de la Espectroscopia de Resonancia Magnética Nuclear ^1H (RMN- ^1H). Se encontraron diferencias en la composición de la mezcla de reacción de las muestras a través del análisis por RMN- ^1H . Durante la ozonización del ácido oleico, los acil-oxi-alquil-hidropéroxidos se formaron como productos mayoritarios en todas las condiciones estudiadas. No obstante, durante la ozonización del oleato de metilo, se encontró una dependencia llamativa de los productos de la reacción con el disolvente o el aditivo usado. En hexano, se obtuvieron los ozónidos/oligómeros correspondientes, considerando que cuando el etanol está presente, los etoxi-hidropéroxidos eran los productos mayoritarios. El análisis en presencia de agua sólo revela la presencia de aldehído y de ozónidos/oligómeros. Estos resultados se discuten teniendo en cuenta el mecanismo de Criegee, las características del reactor de burbujeo empleado y algunas propiedades de los disolventes o aditivos usados.

ABSTRACT: Reactions of ozone with oleic acid or methyl oleate were carried out in presence of hexane, carbon tetrachloride, ethanol or water. All the reaction mixtures were studied by ^1H - Nuclear Magnetic Resonance Spectroscopy (^1H -NMR). Differences in the composition of the reaction mixture of the samples by ^1H -NMR analysis were found. During the ozonation of oleic acid, the acyl-oxy-alkyl-hydroperoxides were formed as major products in all the studied conditions. Nevertheless, during ozonation of methyl oleate, a striking dependence of reaction products with the solvent or additive used was found. In hexane, the corresponding ozonides/oligomers were obtained, whereas when ethanol is present, the ethoxy-hydroperoxides were the major products. The analysis in the presence of water only reveals the aldehyde and ozonides/oligomers signals. These results are discussed taking into account the Criegee mechanism, the characteristic of the bubbling reactor employed, and some properties of the solvents or additives used.

INTRODUCTION

Oleic acid (cis-9 octadecenoic acid) is one of the most important components of vegetable oils and the lipids present in the living organisms.^{1,2} For example, olive oil contains 60-70 % of oleic acid and sunflower oil 20-30 %.³ The oleic acid presents an unsaturation in its structure, and for that reason readily reacts with ozone, at a rate constant of 10^5 - 10^6 L mol⁻¹ s⁻¹.^{4,5} The reaction of ozone with unsaturated compounds takes place through the well-known Criegee mechanism.⁴⁻⁷

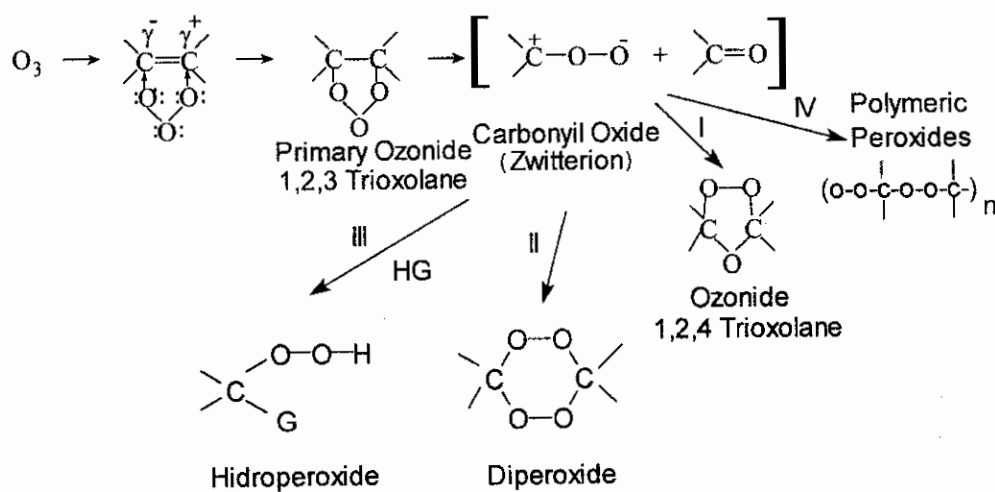
This mechanism (Fig. 1) consists in an ionic, electrophilic attack of ozone, in a simultaneous way, at the carbon-carbon double bond (1,3 dipolar cycloaddition), resulting the 1,2,3 trioxolane or primary ozonide. This intermediate is very unstable and it is decomposed forming a carbonylic compound (aldehyde or ketone) and the carbonyl oxide or zwitterion. In that moment, depending on the reaction conditions, different routes can be taken and different reaction products can be obtained.⁴⁻⁷

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several factor related to reaction conditions to improve or to increase the yield of a specific product. Among these factors are: the use or not of solvents, the type of solvents, the presence and concentration of additives, the reaction temperature, the type of reactor, the agitation of the reaction mixture, etcetera.⁴⁻⁷ In the present case, the composition of the reaction mixture of ozonated oleic acid and its methyl ester, under different reaction conditions were studied. The ¹H-NMR technique has

been used with very good results for the study of this kind of reactions.^{8,9} It is a non-destructive analytical method that does not require of standards in the reaction mixture for component identification.⁶⁻⁸ The corresponding signals from the compounds present in the studied mixtures are very well characterized in the specialized literature.⁶⁻⁹ Other aspect in favour of this technique is that it permits to accomplish an integral analysis of the mixture, without previous purification steps.⁹



MATERIALS AND METHODS

Reactives and Solvents: Analytical grade oleic acid, methyl oleate, sodium sulfate, *n*-methyl-*n*-nitroso-*p*-toluen sulfonamide and tetramethylsilane were purchased from Sigma and they were used without previous purification. Solvents like hexane p.a., carbon tetrachloride p.a. and ethyl ether p.a. were supplied by MERCK; acetonitrile p.a. was supplied by BDH and ethanol was technical grade. Hexane previously ozonated and distilled was used. Ethanol was purified using a fine distillation column before its utilization.

The oleic acid ozonation was carried out in three different conditions:

- in presence of 10% v/v of water (water was used as additive).
- in presence of 10% v/v of ethanol (ethanol was used as additive).
- dissolved in carbon tetra-chloride (0.35 M).

The methyl oleate ozonation was carried out:

- dissolved in hexane (0.35 M).
- in presence of 10% v/v of ethanol

Substrate ozonation in presence of solvents (hexane or carbon tetrachloride): 10 mL of a 0.35 M solution of the substrate was placed in a bubbling reactor, with an oxygen flow of 10 L/h. The reactor was immersed in a water bath at $25.0 \pm 0.1^\circ\text{C}$. It was used an OZOMED-400 ozone generator (Cuba). The ozone dose was determined by measuring the absorbance at 256 nm, in an Ultrospec III spectrophotometer (Pharmacia). The ozone dose applied varied between 50 - 55 mg ozone/g of substrate, with eight runs for each case. In Fig. 2, a schematic diagram of general ozonation process is shown.

Ozonation in presence of water or ethanol (additives employed): A heterogeneous mixture (1:9 v:v) of water or ethanol and substrate was prepared. The conditions and the installation were the same as those of the ozonation in presence of solvents.

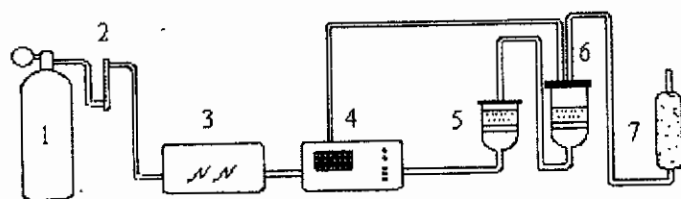


Figure 2. Schematic diagram of general ozonation process.
1- Oxygen source, 2- Bubbling flowmeter, 3- Ozone generator, 4- Spectrophotometer UV-Vis, 5- Solvent or additive vessel, 6- Bubbling reactor, 7- Ozone destructor.

Measurement of ^1H NMR Spectra

The ^1H NMR spectra were obtained with a Bruker AC-250 F spectrometer with Fourier transformed. The samples were dissolved in deuterated chloroform and tetramethylsilane (TMS) was used as internal reference. 5 KHz spectral width, 60 degree pulse width (5 μs), 8 scans, and 32 Kbytes of memory were used to obtain the spectra. It was not necessary to use an internal standard to measure the integrals of

the signal areas because the methoxylic signal of the methyl oleate can be used as reference value for the relationship of the intensity values of each spectrum.

RESULTS AND DISCUSSION

Oleic acid ozonation

Comparing the ^1H -NMR spectra of oleic acid before and after ozonation meaningful differences were found (Fig. 3). The oleic acid spectrum only showed the

corresponding signals of the olefinic protons with chemical shifts between δ 5.3-5.5 ppm and the corresponding signals of the rest of the protons, below δ 3.0 ppm. On the other hand, ^1H -NMR spectra of ozonation mixtures obtained under all the experimental conditions, above described were identical (from a qualitative point of view). In Fig. 3 B, a representative spectrum of reaction mixture is shown:

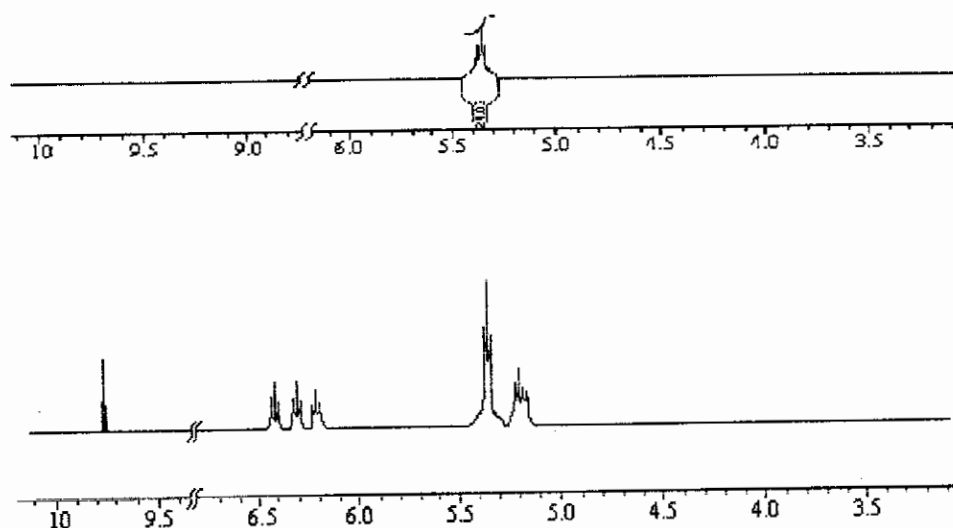


Figure 3. A- ^1H -NMR spectrum of oleic acid, B- ^1H -NMR spectrum of ozonated oleic acid.

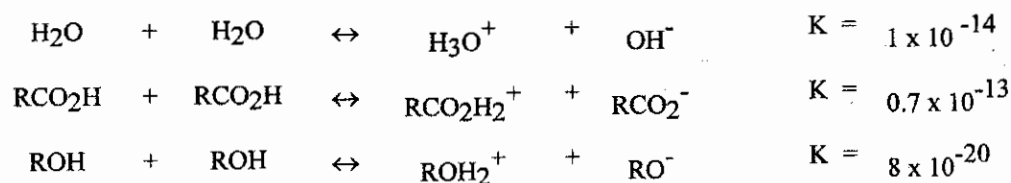
δ 5.3-5.5 ppm - olefinic protons, δ 5.1-5.3 ppm - ozonides/oligomer protons,
 δ 6.1-6.5 ppm - acyloxyalkyl hydroperoxides protons and δ 9.7-9.9 ppm - saturated aldehyde protons

Taking into account the Criegee mechanism, different products in each studied condition could be obtained. The results found in carbon tetrachloride, agreed with the expected ones. Oleic acid is a protic compound and it is capable to interact with the carbonyl oxide, by inter and intra molecular reactions, to form acyloxyalkyl hydroperoxides (signals with chemical shifts between δ 6.1 - 6.5 ppm).⁹ This reaction path is favored in relative

rather high concentration of oleic acid. Under dilute conditions, where oleic acid concentration is rather low, the possibility of forming ozonides increase by recombining reaction of the carbonyl oxide with the aldehyde fragment.⁹

When protic additives (ethanol or water) are used the interaction processes of these additives with the reaction intermediate, to form other peroxidic compounds would be favored. In this case, increase amounts of aldehydes and formation

of ethoxy and hydroxyl hydroperoxides should be expected, modifying substantially the resulting ^1H -NMR spectra. Oleic acid is the most acidic substance, water autoprotolysis constant is only one order lower, but water it is not a good solvent for oleic acid. Ethanol is capable to dissolve the oleic acid, but its acidity is much lower, that is why, at the studied conditions, no ethoxy or hydroxy hydroperoxides were registered.¹¹



Methyl oleate ozonation

Ozonation in hexane:

The ^1H -NMR spectrum, in the range of δ 3-10 ppm (Fig. 4), only showed the formation of ozonides and oligomers, with signals between δ

5.1-5.3 ppm. The signal with chemical shift between δ 5.3-5.5 ppm (a triplet) denotes the existence of vinyl protons, while the signal in δ 3.67 ppm represents the methyl group of the ester.^{12,13}

Comparing the ^1H -NMR spectra of methyl oleate, before and after ozonation, a decrease of the intensity of vinyl proton signals was observed, as well as the appearance of the signals between δ 5.1-5.3 ppm.^{12,13}

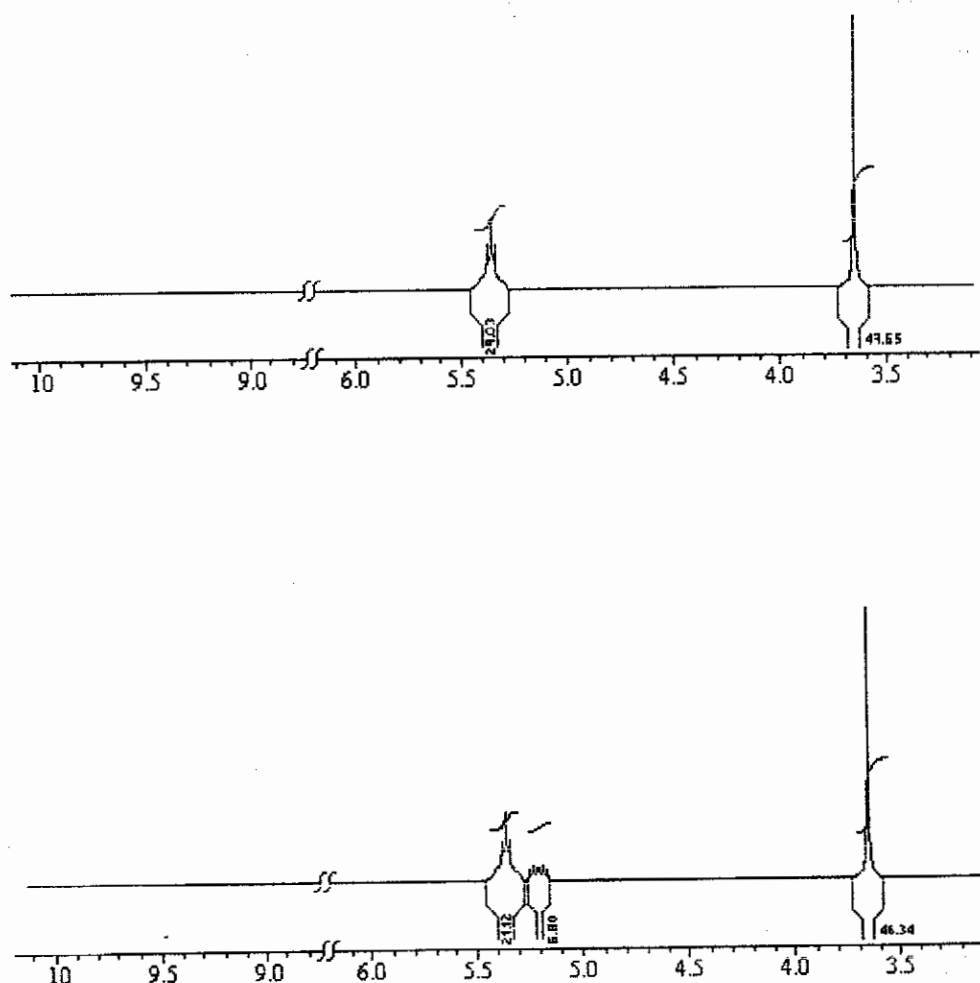


Figure 4. A- ^1H -NMR spectrum of methyl oleate, B- ^1H -NMR spectrum of methyl oleate ozonated in hexane (rango de δ 3-10 ppm).

According to the literature, the signals of the 1,2,4-trioxolane protons appeared between δ 4.85-5.51 ppm.^{6,9} Considering that the protons of the 1,2,4 trioxolane, generated in the methyl oleate ozonation in petroleum ether, shows up at δ 5.1 ppm and that protons of ozonides and oligomers, present similar chemical environment and have the same chemical shift, the signals between δ 5.1-5.3 ppm are attributed to both compounds.^{5,12} It is reasonable that only ozonides and

oligomers appear as reaction products in a non-participating solvent as hexane.^{4,14}

These results are similar to those obtained by Rebrovic,⁹ in methyl oleate ozonation in iso-octane and by Pryor, in methyl oleate ozonation in carbon tetrachloride and hexane.^{5,12} Analyzing the corresponding signals of ozonides it is observed that apparently it is a quintuplet, being in reality two triplets, those represent the respective cis-trans isomers.⁹

Ozonation in ethanol

The ^1H -NMR spectrum obtained for methyl oleate ozonation in ethanol (0.35 M) is shown in Fig.5. The formation of ethoxy hydroperoxides (δ 4.8-4.9 ppm) as major products is corroborated.

The ozonides/oligomers (δ 5.1-5.3 ppm) and the aldehyde signals δ 9.7-9.8 ppm are also observed, but in smaller proportions.

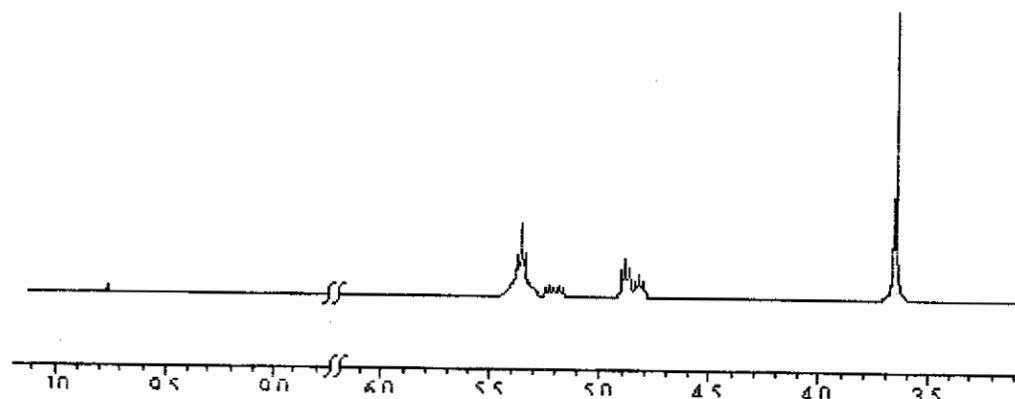


Figure.5. ^1H -NMR spectrum of methyl oleate ozonated in ethanol.

Obviously ethanol as a protic solvent, favorably competes in the reaction with the carbonyl oxide or zwitterion intermediate.⁴ Nevertheless, the ozonides/oligomers formation is also meaningful under our experimental conditions.

Ozonation in presence of water:

While in methyl oleate ozonation in hexane, only ozonides/oligomers were obtained and the ozonation in ethanol rendered a mixture of ethoxy hydroperoxides and ozonides, the reaction of methyl oleate with

ozone, in presence of water, mainly yielded aldehydes. Although a small proportion of ozonides/oligomers were also found. The spectrum obtained by ^1H -NMR is shown in Fig. 6.

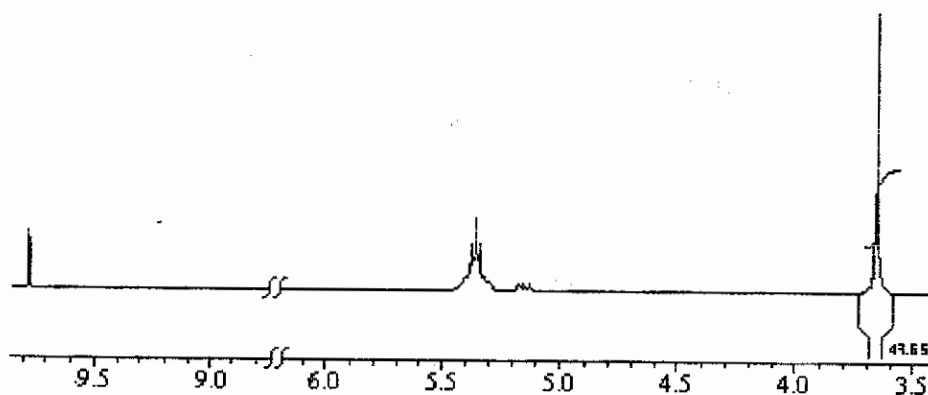


Figure. 6. ^1H -NMR spectrum of methyl oleate ozonated in presence of water

According to the Criegee mechanism, water can interact with the zwitterion to form hydroxy hydroperoxides. These hydroperoxidic compounds are very unstable and they readily decompose and produce mainly aldehydes and hydrogen peroxide.^{4,15}

CONCLUSIONS

In all the studied conditions, the Criegee mechanism can explain the formation of ozonation products. The ozonation products of oleic acid in the studied conditions were acyloxyalkyl hydroperoxides, ozonides/oligomers, and aldehydes, independently of the system under study. It was explained taking into account the higher acidity and concentration of oleic acid respect to water or ethanol used as additives. At the 10 % (v/v) additives concentration in the oleic acid ozonation there was not observed any change by ¹H-NMR in the final products composition.

The methyl oleate ozonation products depend on the polarities of solvents and/or additives present in the reaction mixture. Subsequently ethoxy hydroperoxides were obtained when ethanol was present. High amounts of ozonides/oligomers, and aldehydes were found in presence of water or dissolved in hexane.

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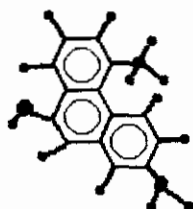
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