

Kinetic Study on the Autoxidation of Methyl Linolenate by NMR Spectrometer

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NMR에 의한 Methyl Linolenate의 자동산화속도測定

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초 록

自動酸化反應의 Model化合物로서 methyl linolenate를 時間別로 상온과 90ml/min의 air flow rate下에 96時間까지 酸化시켰다. 이들 酸化反應物에 對한 NMR測定值로부터 反應速度常數와 反應次數를 算出한 結果 methyl linolenate의 自動酸化는 一次 反應速度論을 따르며, 그 反應速度常數는 $1.96 \times 10^{-2} \text{hr}^{-1}$ 이었다. 脂肪酸의 自動酸化에 對한 Kinetics研究에 여러가지 方法이 利用되고 있으나 本實驗結果로 보아 NMR가 보다 迅速하고도 効果的인 分析方法이라고 생각된다.

Introduction

Triunsaturated fatty acids are present as a major acid in a large number of vegetable oils and display pronounced biological activities.^{1,2)} Early reports have pointed to linolenic acid as the unstable precursor of reversion flavors in linolenate-containing soybean, marine, rapeseed, and perilla oils. By contrast, however, corn, cottonseed, peanut and olive oils, which are free of linolenic acid, are said to develop rancid flavors on storage.^{3,4)} The oxidation of polyenes

is initiated more readily and proceeds more rapidly than that of monoenes. The greater the number of double bonds, the greater the tendency to oxidize.⁵⁾ The oxidation rates of purified oleate, linoleate and linolenate esters were reported by Hammond et al. It was suggested that methyl lineate oxidized about ten times faster than methyl oleate and methyl linolenate two times faster than methyl linoleate.⁶⁾

A number of analytical methods have been used for kinetic study on the autoxidation of unsaturated fatty acids.⁷⁻¹¹⁾ Nevertheless, in a few cases NMR spectrometry has been utilized as an analytical tool.^{12,13)} The purpose of this study was to evaluate the applicability of NMR spectrometer to kinetic study on the autoxidation of methyl linolenate as a model compound.

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Materials and Methods

All of the reagents used in this experiment were special grade and purchased from Merck Company, if not specified. The materials used were methyl linolenate(99% pure, Tokyo Kasei Co.; iodine value of 237), tetramethylsilane (TMS), chloroform, and carbon tetrachloride.

The iodine value of methyl linolenate was determined by the method of AOAC-Wijs.¹⁴⁾ Methyl linolenate (30mg each) was placed in each of six NMR tubes attached to a capillary tube and aerated at the flow rate of 90ml/min by using air compressor. The aeration was continued at room temperature for the periods of 16, 32, 48, 64, 80, and 96hrs. To the autoxidized methyl linolenate placed in NMR tube, were added TMS(2%), chloroform(3%) and carbon tetrachloride(350 μ l) by using automatic pipet, followed by measuring the autoxidation rate by NMR spectrometer (Varian T-60, 60MHz).

The decrement of peak height at 5.38ppm was compared with the increment of peak height at 7.10ppm which corresponds to the chemical shift of chloroform added as an internal standard. The concentration of unreacted methyl linolenate was calculated from the ratio of the two peak heights, and the rate constant and reaction order were determined by the differential method of Van't Hoff¹⁵⁾ ($V=KC^n$), where V expresses the reaction velocity, C represents the concentration of unreacted methyl linolenate, n denotes the number of order, and K expresses

the rate constant.

Results and Discussion

The NMR spectrum of methyl linolenate was shown in Fig. 1, and the chemical shift and number of protons were assigned as shown in Tale I.

Much evidence has been introduced to suggest that the autoxidation of fats proceeds via typical free radical mechanisms.¹⁶⁻¹⁹⁾ In the initial step of the autoxidation of unsaturated fatty acids, free radical is formed at active methylene groups by abstracting hydrogen and reacts with molecular oxygen to give hydroperoxide. This hydroperoxide breaks down in several steps yielding a wide variety of decomposition products which reduce in turn the number of the vinyl protons of unsaturated fatty acids. The case was found in this experiment; as shown in Fig. 1 and 2, the peak height of vinyl proton at 5.38ppm was reduced as the autoxidation proceeds. No more reduction of the peak height was observed after 96hrs. Thus, the experimental results certainly encouraged us to carry out kinetic study on the autoxidation of methyl linolenate by using NMR spectrometer.

The ratio of the peak height at 5.38ppm to the peak height of chloroform as an internal standard at 7.10ppm was calculated. The ratio was then converted to the millimolar concentration of each sample obtained from a definite reaction time, as shown in Fig. 3. The autoxidation was very slowly proceeded from 0 to

Table 1. Peak assignments and proton count of NMR spectrum for methyl linolenate (TMS peak at 0ppm and CHCl_3 peak at 7.10ppm)

Chemical shift, ppm	No. of Protons	Partial Structure
0.97	3	$\text{CH}_3-\text{C}-\text{C}=\text{C}-\text{C}-$
1.33	10	$-\text{C}=\text{C}-\text{C}-\text{CH}_2-\text{C}-\text{C}=-$
2.25	6	$\text{C}-\text{CH}_2-\text{C}=\text{C}, =\text{C}-\text{CH}_2-, \text{C}-\text{CH}_2-\text{C}=\text{O}$
2.80	4	$\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}-$
3.67	3	$\text{O}=\text{C}-\text{O}-\text{CH}_3$
5.38	6	$-\text{C}-\text{CH}=\text{CH}-\text{C}-$

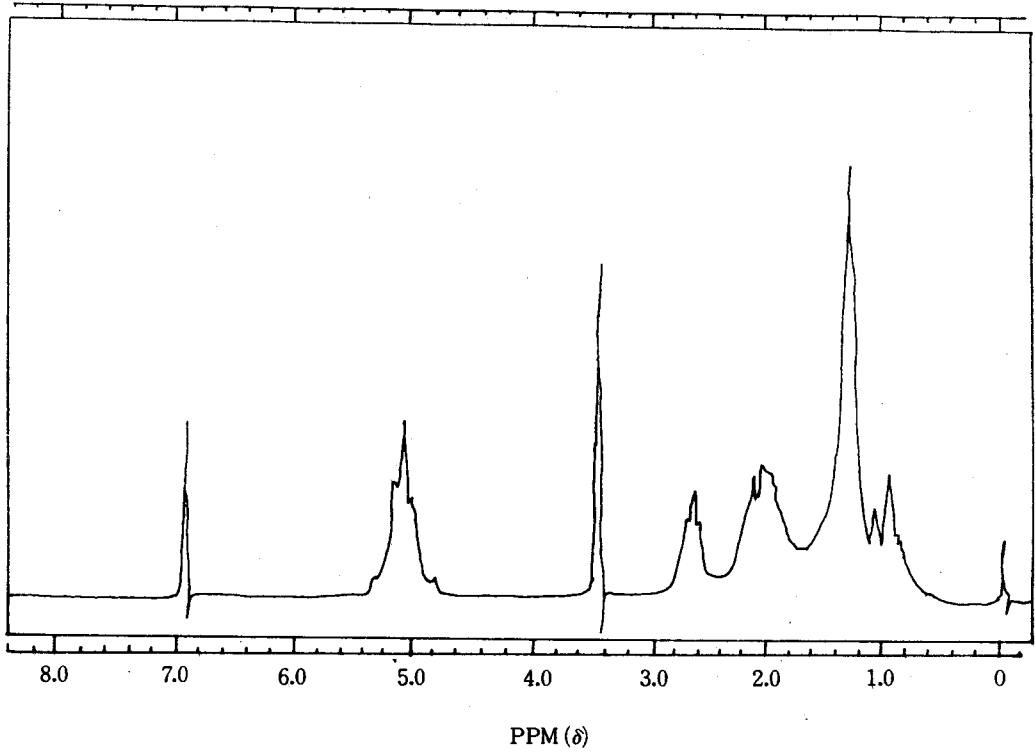


Fig. 1. NMR spectra of methyl linolenate

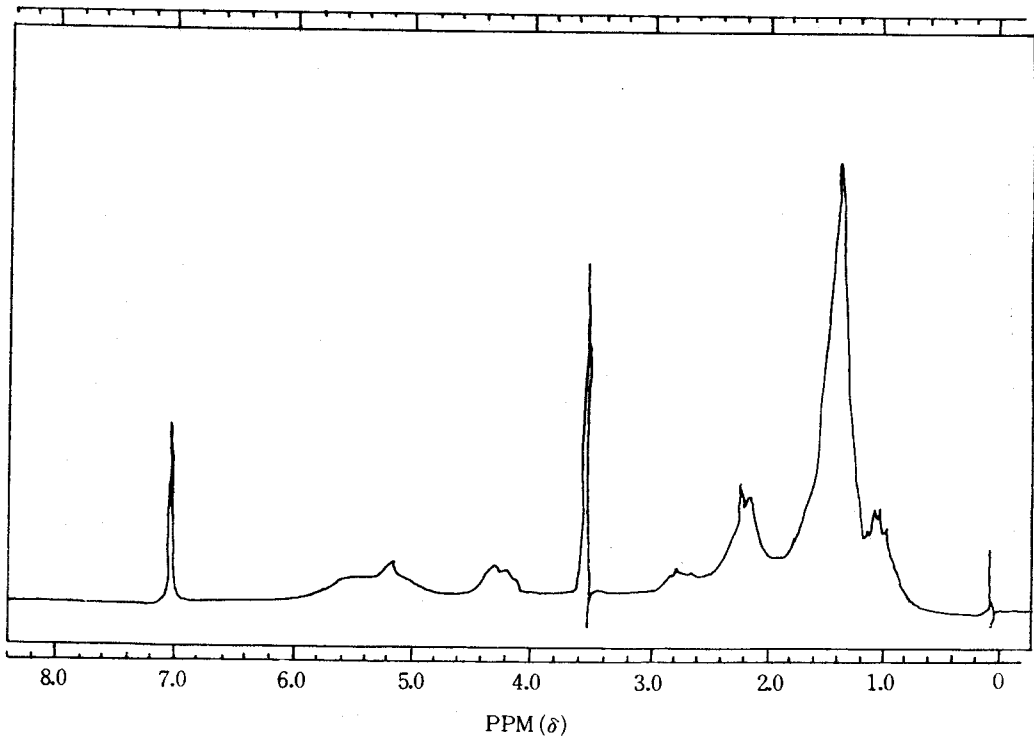


Fig. 2. NMR spectra of methyl linolenate aerated 96hr at 25°C

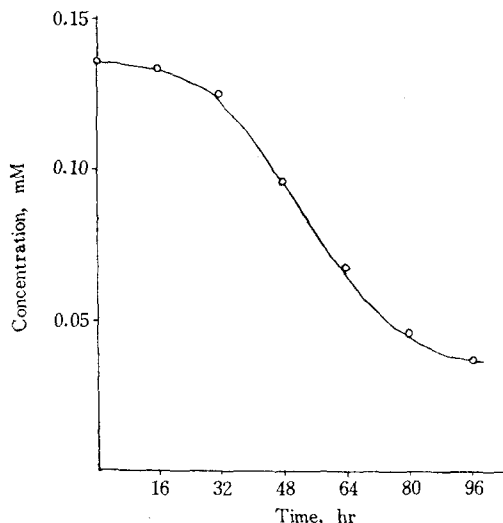


Fig. 3. Concentration(mM)-time curve of aerated methyl linolenate

32 hrs, indicating a relatively long induction period which is usually observed from the autoxidation of pure substrate.

According to the differential method of Van't Hoff,¹⁵⁾ the concentration of unreacted methyl linolenate at each reaction time, $A_0 - X$ or C , against reaction time was plotted, and the concentration multiplied by 100 to be positive log value as shown in Fig. 4, where A_0 expresses the initial concentration of methyl linolenate and X represents the the concentration of the substrate reacted. The slope (i.e., a velocity V) for each reaction time was calculated from the curve, and $\log V$ was again plotted against $\log C$ at each reaction time to obtain the slope of 1.0 as shown in Fig. 5. Following the first-order kinetics, the rate constant was found to be $1.96 \times 10^{-2} \text{hr}^{-1}$. This agrees very well with the results reported by Hendriks et al.¹⁹⁾ Quencer et al.¹¹⁾ oxidized methyl linolenate at the flow rate of 5 liter O_2 per minute and 91°C for 5 min and measured the oxygen absorption rate by using manometer. They found that the rate constant and reaction order were 0.310min^{-1} and 0.6, respectively. It was reported by Mabrouk et al.⁹⁾ that linoleic acid and its ester

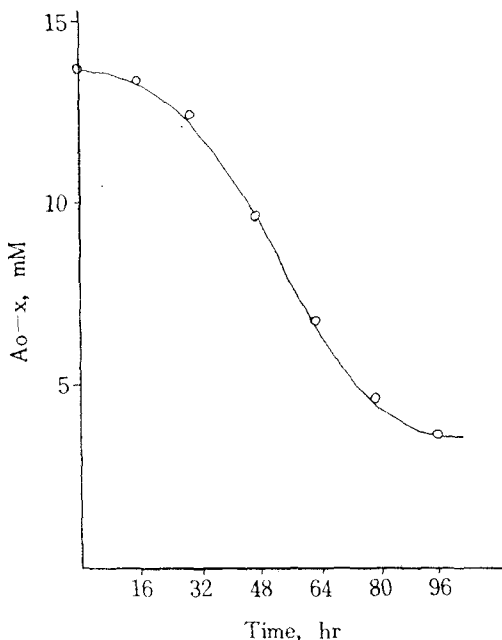


Fig. 4. Reactant concentration-time curve in the autoxidation of methyl linolenate

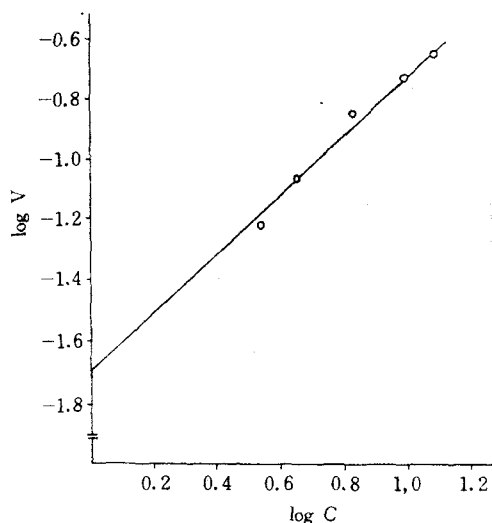


Fig. 5. A plot of $\log V$ vs. $\log C$ for the determination of number of order in the autoxidation of methyl linolenate

showed different oxidation rates and that linoleic acid oxidized more rapidly than methyl linoleate.

In view of the fact that naturally occurring fatty acids are not usually found in ester form,

the rate constant of the autoxidation of linolenic acid in the biological system might be somewhat greater than that of methyl linolenate. In conclusion, it should be noted that nuclear magnetic resonance spectrometer can be efficiently utilized as an analytical tool for kinetic study on the autoxidation of polyunsaturated fatty acids.

Abstract

The rate constant and reaction order of the autoxidation of methyl linolenate were determined by using nuclear magnetic resonance spectrometer, and the applicability of nmr to the kinetic study was examined. The autoxidation was carried out under a mild condition, i.e., the air flow rate of 90ml/min and room temperature. The autoxidation of methyl linolenate followed the first-order kinetics, and the rate constant was found to be $1.96 \times 10^{-2} \text{hr}^{-1}$.

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