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Examination of Fat Oxidation Products by FT-NMR & FT-IR

De Chen

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1993

EXAMINATION OF FAT OXIDATION PRODUCTS
BY FT-NMR AND FT-IR

A Thesis

Presented to

the Faculty of the Department of Chemistry

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

De Chen

May 1993

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EXAMINATION OF FAT OXIDATION PRODUCTS
BY FT-NMR AND FT-IR

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ACKNOWLEDGMENTS

I wish to express my sincere gratitude and deep appreciation to my advisor, Dr. David R. Hartman, for his help with the experiments and in preparing the manuscript. His dedication and professional input made my two years of graduate work enjoyable.

I am grateful to the members of my graduate committee, Dr. John W. Reasoner and Dr. Thomas K. Green for their help throughout the duration of my studies.

Appreciation is extended to Dr. Robert W. Holman for teaching me how to operate NMR instrument and to Ms. Jean Almand for library assistance.

I would also like to thank my wife, Yijuan Liu, and my parents, because without their help and support none of this would have been possible.

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EXAMINATION OF FAT OXIDATION PRODUCTS BY FT-NMR AND FT-IR

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May 1993

33 Pages

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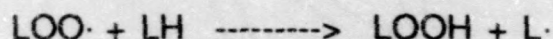
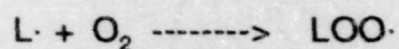
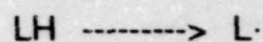
Edible oils are easily oxidized when used for frying. Oxidation originates with double bonds present in unsaturated fatty acids. A new NMR method is presented which offers potential for determining the products of oil oxidation. High resolution fourier transform nuclear magnetic resonance (FT-NMR) and fourier transform infrared (FT-IR) spectroscopy have found increasing use in biochemistry. One pure fat, trioleoylglycerol, was heated at 160°C in the presence of air. Samples were taken for FT-NMR and FT-IR analyses at 4, 10, 20, 25, 35, 50, 70 and 100 hours. Proton, Carbon-13 and several types of two-dimensional FT-NMR spectra were obtained using a JEOL 270 Mhz instrument. The spectra suggest initial oxidation occurs by an allyl free radical mechanism facilitating the formation of epoxide and peroxide products.

INTRODUCTION

Fats and oils are a major source of calories in the American diet. Fat or oil frying is a method of cooking commonly used for the manufacture and preparation of foods. The fat serves as a heat-transfer medium, as an important ingredient of the fried food, and provides flavor for foods, energy and essential fatty acids. Edible oils are easily oxidized when heated or fried because the natural oils and fats usually contain unsaturated fatty acids. Research has shown that when a fat or oil is heated extensively, new compounds are formed and the ingestion of these products may be harmful to the health of animals. The idea that some of the products of *in vivo* lipid oxidation are deleterious to human health is not new. However, the concept that dietary lipid oxidation products and thermally altered lipids are injurious has attracted much interest recently and is the subject of much research [1].

As is well-known, the most important cause of the oxidative deterioration of lipids is the dissolution of oxygen from air and its subsequent reaction with the unsaturated glycerides. Several types of oxidized compounds are formed. It is well known and accepted that primary oxidation products of lipids are hydroperoxides [1]. These compounds form relatively rapidly and then react further to yield a variety of secondary and subsequent products: peroxides, aldehydes, ketones, and acids.

In the presence of an initiator, unsaturated lipids (LH) form carbon-centered alkyl radicals (L·) and peroxy radicals (LOO·), which propagate in the presence of oxygen by a free radical chain mechanism to form hydroperoxide (LOOH) as the primary product of autoxidation [2].



In the presence of light, unsaturated fats can also form hydroperoxides by reacting with single oxygen produced by sensitive photooxidation, which is a non-free-radical process [3].

Lipid hydroperoxides are readily decomposed into a wide range of carbonyl compounds, hydrocarbons, ketones and other materials that contribute to flavor deterioration of foods. The volatile oxidation products of unsaturated lipids cause rancidity in foods and cellular damage in the body [4].

The oxidation of a fat at high temperatures, as when fats are used in frying, combines the effect of both oxygen and temperature on fats. During frying of foods, the oil or fat is kept at elevated temperature and exposed to air, water and the chemical components of the food being fried. These conditions lead to thermal, oxidative and hydrolytic decomposition of the fat [5]. Heating oils to high temperatures greatly accelerates the autoxidative process and

through a complex series of reactions produces numerous decomposition products. The extent and nature of decomposition products are affected by frying parameters such as fat and food composition, frying conditions (temperature, oxygen exposure, heating time, turnover rate), and the design and construction material of frying equipment which differ from one place to another.

One of the most important changes taking place when a fat is heated to high temperature is the formation of polymers. Polymer formation is accompanied by an increase in viscosity, a decrease in iodine value, darkening of the oil, an increase in saponification value and an increase in foam formation. Many of the changes observed in heated fats are the direct result of the chemical incorporation of oxygen into the oil and hydrolysis of the triglycerides. Consequently, the progress of frying oil deterioration has also been followed by measurements of changes in specific functional groups such as peroxides, carbonyls, conjugated double bonds, and free fatty acids [6].

The free fatty acids are formed by hydrolysis of triglycerides, which is promoted by the presence of food moisture and by oxidation, or by the reaction of oil with moisture formed during other deterioration reactions. The other compounds (peroxides, carbonyl, conjugated double bond) are formed by various reactions with oxygen. The hydroperoxide was normally formed first and peroxide value measures hydroperoxide. Peroxide value may be less reliable for monitoring thermal deterioration because of the rapid decomposition of peroxides that are formed during primary oxidation. The increase in the absorbance at 232 nm and 268 nm

indicated the formation of conjugated compounds (dienes and trienes) from linoleic and linolenic acids due to the shift of the double bond during frying [7]. Frying of food resulted in the darkening of oils because of oxidation and of the colored pigments from the foods which diffused into the oil during frying. The increase in viscosity during fat frying was due to polymerization, which resulted in the formation of higher molecular weight compounds (carbon-to-carbon and/or carbon-to-oxygen-to-carbon bridges between fatty acids) [8].

Health concerns have been raised regarding heated fats from the inception of deep-frying. Both saturated and unsaturated triacylglycerols undergo both oxidative and nonoxidative (thermolytic) degradation. Saturated fats are more stable, but potentially significant thermolytic and oxidative products can occur, particularly in abused oil. Unsaturated fats degrade rapidly, although hydrogenated fats somewhat less so [9].

When fats and oils are heated in the presence of air, a number of metabolically active substances are produced. All these products of fat oxidation are toxic. Feeding of rats with fats oxidized at low temperature (10-20% of the diet) caused a loss of appetite, growth reduction and enlargement of the liver and kidney [10]. Similar results were also observed when swine were fed with the oxidized fats [10].

Thermally oxidized fats and oils produce more adverse effects than their unheated counterparts. The extent of adversity varies with the heat treatment given to fats and oils.

Fried foods are a major source of dietary oxidation products. Clearly, more research is needed on the detailed content of all the various lipid oxidation products in such foods. An assessment is needed of the toxicology of these oxidation products using appropriate animal models, relevant combinations and levels of oxides and appropriate experiment treatment durations. In addition, a similar assessment is needed of the dimers, trimers and polymers produced by the thermal degradation of fats. There appears to be an urgent need for research on improved methods of monitoring oil quality and improved handling and filtration (clear-up) of oils and shortenings used for deep-frying [13].

The other health-related research in relation to lipid oxidation products includes studies on the possible carcinogenic effects, the long-term effects on membranes, the possible accumulation in tissues, the enzyme effects and the possible clinical manifestation of these phenomena. Progress will likely be slower than in coronary heart disease research because less is known about cancer, aging and other diseases which might be adversely affected by lipid oxidation products.

Frankel, E. N. and Neff, W. E [11,12] have made significant advancements in the investigation of lipid autoxidation. Trilinoleoylglycerol (1.0 g) was autoxidized at 40°C to predetermined levels with pure oxygen in a 1 × 15 cm test tube to a peroxide value (PV) of 125 for kinetic studies and to a PV of 2950 for structural studies [11]. Trilinolenoylglycerol was also examined [12]. The hydroperoxides and secondary products were isolated and characterized to clarify their contribution to oxidative deterioration

of edible oils. The hydroperoxide products were purified by HPLC and identified as intact triacylglycerols by UV, IR, ^1H NMR and ^{13}C NMR analyses and after derivatization by lipolysis, GC, and GC-MS spectrometry. The main primary products included mono-, bis- and tris-9-hydroperoxy-*trans*-10,*cis*-12-; 9-hydroperoxy-*trans*-10,*trans*-12; 13-hydroperoxy-*cis*-9,*trans*-11; and 13-hydroperoxy-*trans*-9,*trans*-11-linolenoyl glycerols. HPLC showed that the mono-hydroperoxides were the only products formed initially. The bis- and tris-hydroperoxides were formed from the monohydroperoxides during autoxidation at $\text{PV} > 18$. Hydroperoxides of linoleate triacylglycerols may be important precursors of volatile compounds contributing to off-flavors of edible oils.

The oxidation products of trilinoleoylglycerol were further characterized by GC and GC-MS analyses of the trimethylsilyl (OTMS) ethers. The secondary oxidation products of trilinoleoylglycerol were identified by GC-MS of derivatives obtained after reduction with sodium borohydride or after hydrogenation. After hydrogenation, silylation and transmethylation, the three secondary products formed, respectively were methyl 9,13-diOTMS stearate, 9 or 13-mono-OTMS stearate, and stearate in a ratio of 1:1:1; methyl-9,13-diOTMS stearate, epoxystearate, and stearate in a ratio of 1:1:1; and methyl 9,13-diOTMS stearate and stearate in a ratio of 2:1. The three secondary oxidation products indicated were bis-(dioxygenated linoleoyl) (mono-oxygenated linoleoyl); monolinoleoylglycerol: bis-(di-oxygenated linoleoyl)-(mono-epoxyene linoleoyl)-monolinoleoylglycerol; and bis-(dioxygenated linoleoyl)-monolinoleoylglycerol, respectively. Other minor

secondary products, identified by TLC and GC after sodium borohydride reduction and transmethylation, included fatty acids containing mono-keto diene, monoepoxyhydroperoxyene, and 9,13-dihydroxy-epoxyene. Dimer or polymer formation in either highly oxidized samples of trilinolein (PV 2950) or further oxidized mono-hydroperoxides of trilinoleoylglycerol was not evident.

MATERIALS AND METHODS

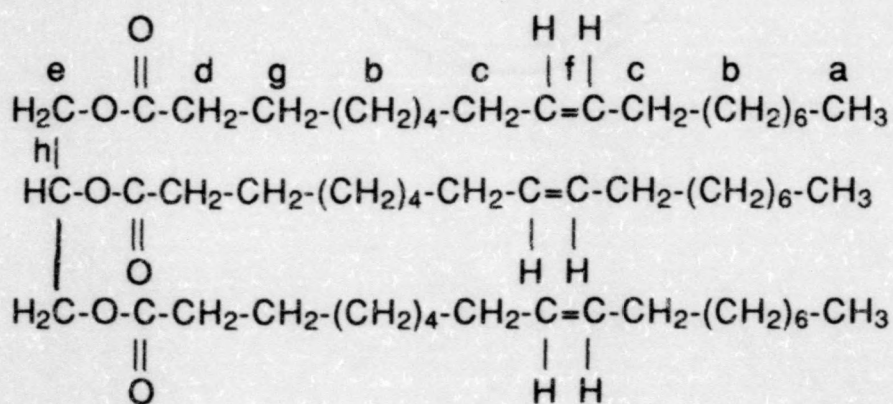
Trioleoylglycerol (Sigma grade, 99%) was purchased from Sigma Chemical Co. (St. Louis, MO 63178). Chloroform (99.8 atom % D)(CDCl₃) +0.05% tetramethylsilane (TMS) was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI 53233).

Trioleoylglycerol (60 mg) was thermooxidized in an oven (Fisher Isotemp Vacuum Oven Model 281) at 160°C with high pressure air, 500 psi, blown over the sample in a 1.0 cm diameter × 3.0 cm high vial. Samples were treated for 4, 10, 20, 25, 35, 50, 70 and 100 hours.

Each was analyzed by NMR using Proton, Carbon-13, and several types of two-dimensional spectra: distortionless enhancement by polarization transfer (DEPT), H-H correlation spectroscopy (COSY) and heteronuclear correlation spectroscopy (HETCOR) immediately in CDCl₃ with a JEOL CPF-270 FT-NMR instrument. All trioleoylglycerol samples used for NMR contained 55-60 mg of oil and 0.7 ml chloroform reagent, and data for 32 scans were averaged. A trioleoylglycerol blank and an oxidized sample (35 hours) were further examined by FT-IR spectrometry (Perkin Elmer 16 PC).

RESULTS AND DISCUSSION

Acceptable spectra were obtained for unheated trioleoylglycerol and are shown in Figure 1 to 5. The ^1H NMR spectrum of the unheated trioleoylglycerol is shown in Figure 1. Characteristic signals for the trioleoylglycerol include 0.9 ppm for CH_3 ; 1.3, 1.6, 2.0 and 2.4 ppm for protons on carbons b, g, c and d of CH_2 ; 5.35 ppm for protons of the double bond carbons f; 4.1, 4.3 ppm and 5.3 ppm for protons of glycerol. ^{13}C NMR data (Fig. 2) showed the trioleoyl carbonyl (173.1, 172.7 ppm) in a 2:1 ratio for 1(3)- to 2-glycerol substitution. Signals were observed for the carbons of the double bond (129.9, 129.6). The signals at 68.8 and 62.0 ppm are due to carbons of glycerol; $-\text{CH}_2-$ signals were located at 34.1 to 22.6 ppm. The signal at 14.0 ppm is due to $-\text{CH}_3$. Figure 2 contains a ^{13}C NMR spectrum indicating 20 different kinds of carbons found in the trioleoylglycerol structure. Using Figure 1 to Figure 5, all of hydrogens in the structure are marked as follows:



The spectra of trioleoylglycerol heated at 10 hours are shown in Figure 6 to 10. Comparing Figure 6 with Figure 1, peak f (5.35 ppm, [-CH=CH-]) and peak c (2.0 ppm, [allyl hydrogens]) become smaller, and there were new peaks at about 1.5 ppm (-CH₂-) and between 2.6 ppm (-CH-C=O) and 2.9 ppm (-CH₂-O-). The evidence indicates that the double bond of the oil is sensitive to thermooxidation.

The ¹H NMR spectra for trioleoylglycerol oxidized for 35 hours are illustrated in Figure 11 to 15. Comparing Figure 1 to Figure 11, peak f and peak c are much smaller in Figure 11 than those in Figures 1 or 6. Several new peaks (-CH₂, -CH-C=O, -CH₂-O-), found in Figure 6 to be larger, were observed in Figure 11, indicating further oxidation in 35 hours.

Utilizing ¹³C NMR, 39 kinds of carbons were identified in oxidized trioleoylglycerol (Fig. 12) compared to only 20 in the blank (Fig. 2). A new peak at 128.0 ppm on the ¹³C NMR spectra (Fig. 12) suggests that the double bond shifted. Two allyl radical resonance forms are indicated. Additional new peaks at 58.6 and 58.7 ppm suggest the presence of peroxide or epoxide carbons. Two-dimensional NMR(DEPT) (Fig. 15) showed these carbons as -CH-. Unoxidized oil (Fig. 5) did not have the peaks at 58.7, 58.6 and 128.0 ppm (new -CH).

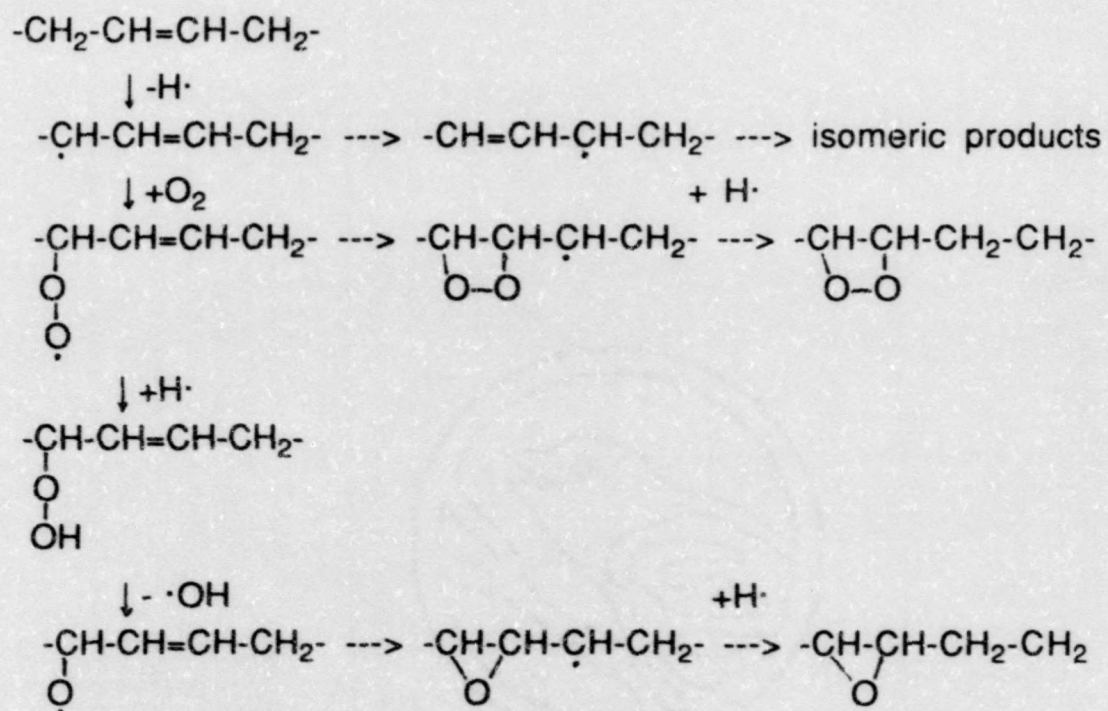
The FT-IR spectra on a blank (Fig. 16) show absorption corresponding to about 3000 cm⁻¹ (olefinic-H), about 1700-1800 cm⁻¹ (carbonyl), and about 1100-1200 cm⁻¹ (C-O). The FT-IR spectra on heated trioleoylglycerol are shown in Figure 17. Comparing Figure 16 with Figure 17, a new peak absorption at about 3500 cm⁻¹

was detected, indicating the presence of hydroperoxide or hydroxyl in the oxidized sample of trioleoylglycerol. The peaks at 1700 cm^{-1} and 3000 cm^{-1} are slightly smaller, indicating a smaller concentration of double bonds in the oxidized oil.

The three ^1H NMR spectra (Fig. 1, 6, 11) of trioleoylglycerol shows the absorption at 2.0 and 5.35 ppm getting smaller ($-\text{CH}=\text{CH}-/-\text{CH}_2- = 3.35/28.96 > 2.71/33.23 > 1.27/30.20$ and $-\text{CH}_2-/-\text{CH}_2- = 5.83/28.96 > 4.89/33.23 > 1.96/30.20$), which indicates a decrease in double bonds (peak f) and allyl hydrogen (peak c) as oxidation proceeds. The longer time of oxidation of the oil, the more oxidized products appear at 1.55, 2.65 and 3.0 ppm ($-\text{CH}_2$, $-\text{CH}-\text{C}=\text{O}$, $-\text{CH}_2-\text{O}-$).

Carbon-13 NMR spectra on heated trioleoylglycerol show absorption indicating new peaks appearing at 58.7, 58.6 and 128.0 ppm which is evidence for peroxide or epoxide and double bond shift, respectively.

FT-IR spectra on heated trioleoylglycerol show absorption that indicates a new peak occurs at about 3500 cm^{-1} . This indicates the presence of $-\text{OH}$ or $-\text{OOH}$. The products of thermooxidation are peroxide or epoxide and hydroperoxide. According to the spectra of 1-D, 2-D NMR and FT-IR, the mechanism of the trioleoylglycerol thermooxidation is proposed:



The identical reactions would occur on the other side of double bond.

Figure 1. ^1H NMR spectrum of trioleoylglycerol.

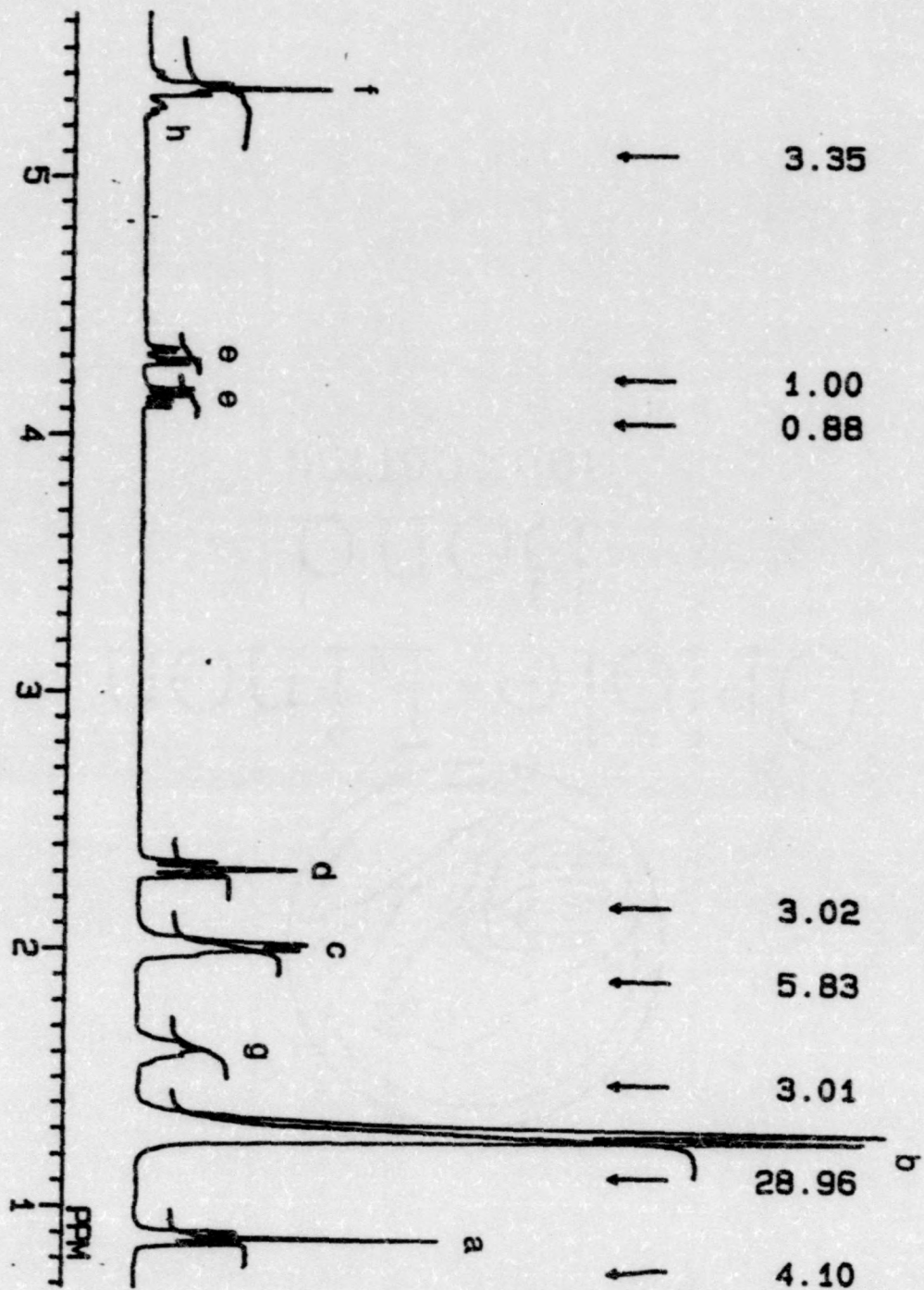


Figure 2. ^{13}C NMR spectrum of trioleoylglycerol.

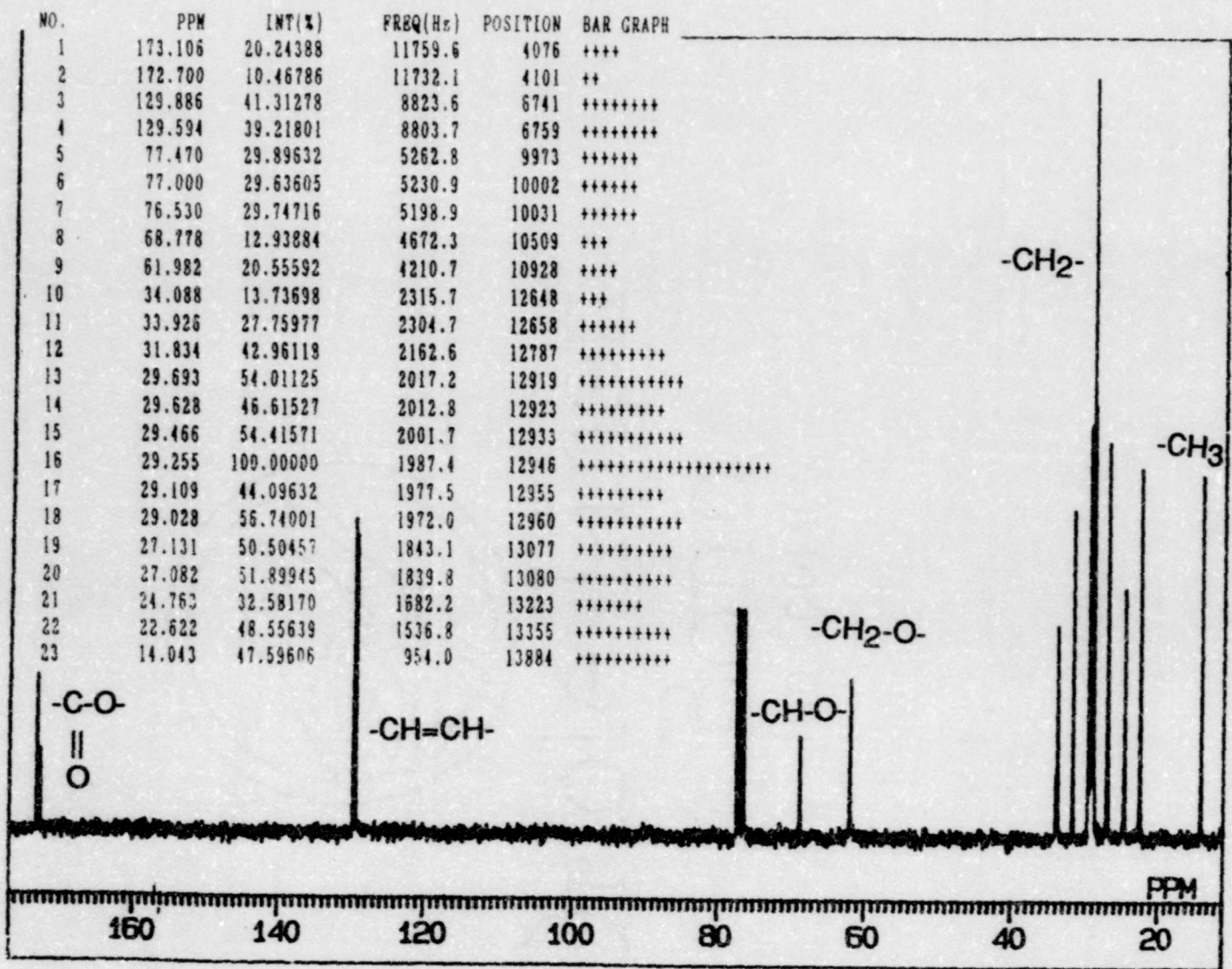
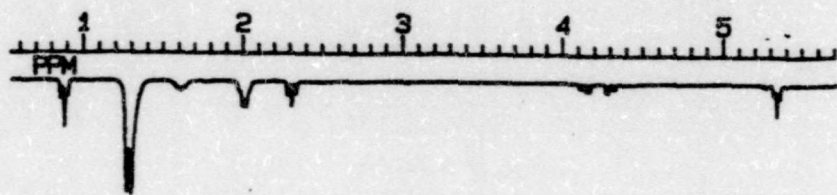
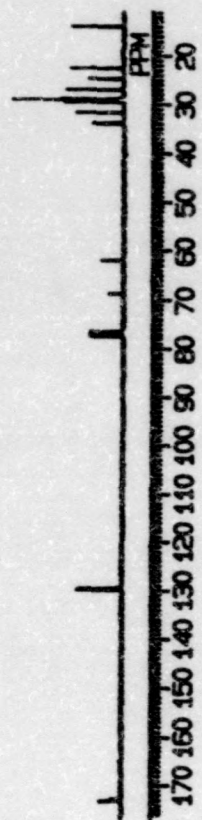
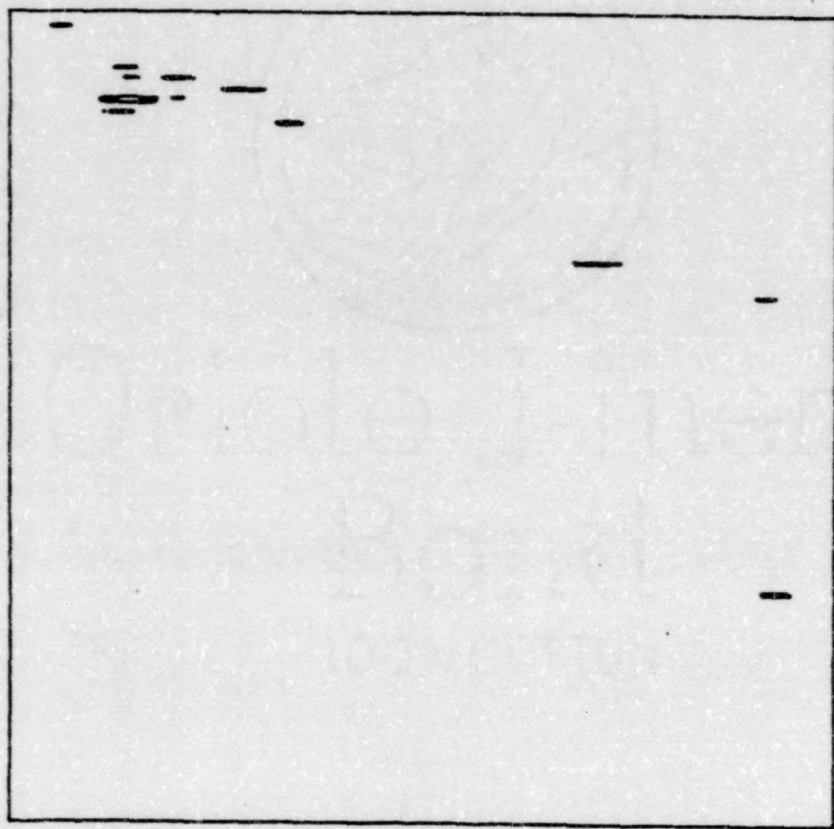


Figure 3. Two-dimensional NMR (HETCOR) spectrum of trioleoylglycerol.



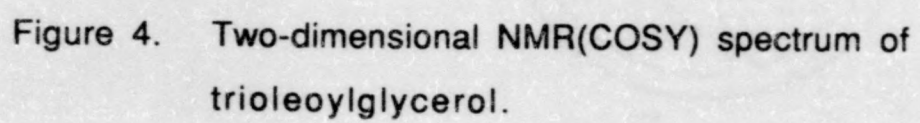


Figure 4. Two-dimensional NMR(COSY) spectrum of trioleoylglycerol.

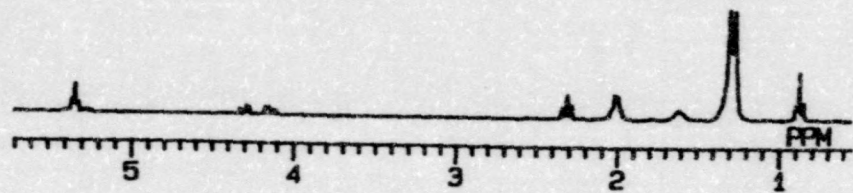
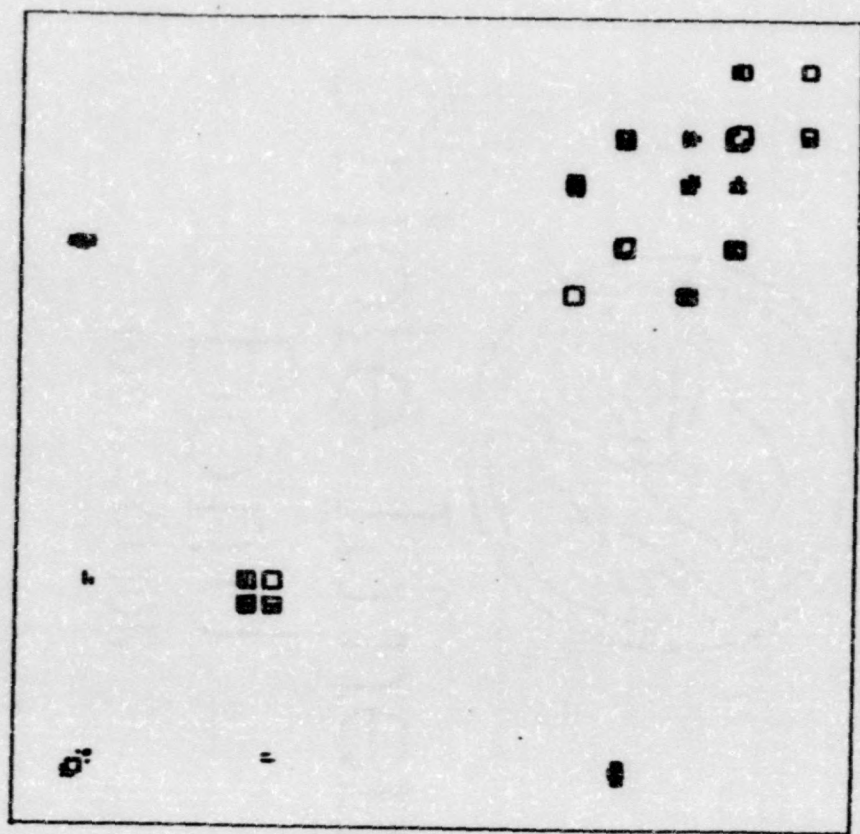
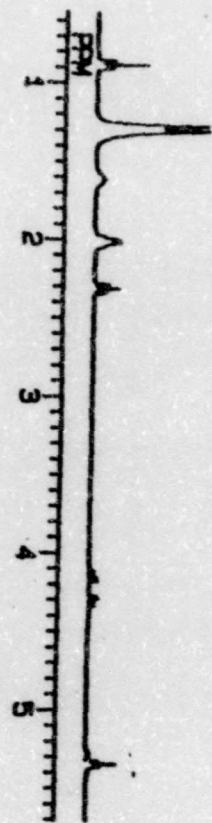
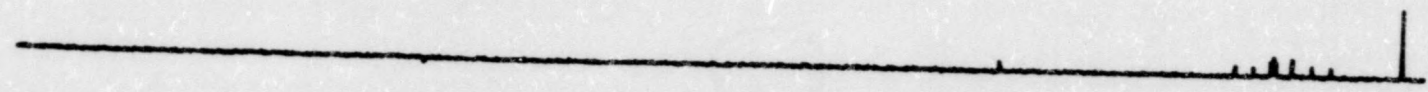
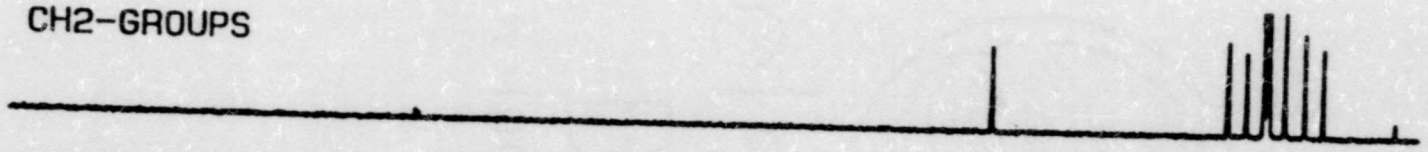


Figure 5. Two-dimensional NMR(DEPT) spectrum of trioleoylglycerol.

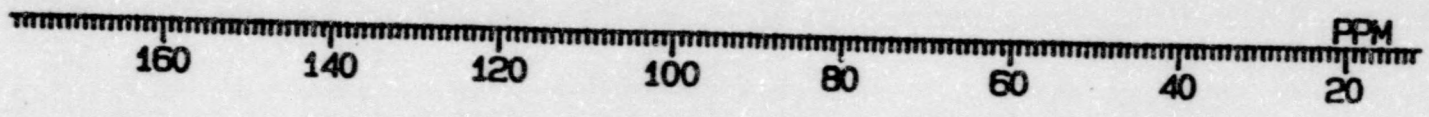
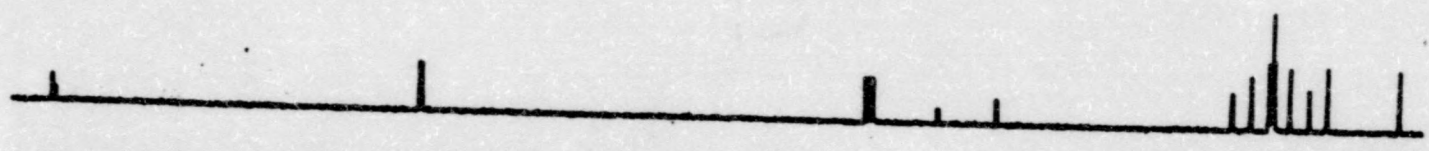
CH3-GROUPS



CH2-GROUPS



CH-GROUPS



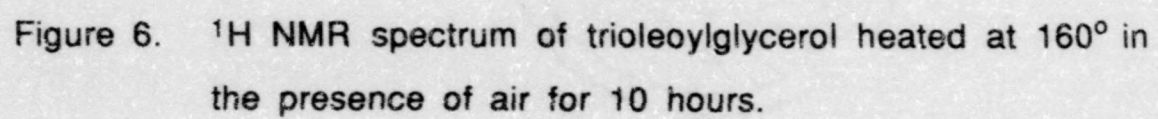
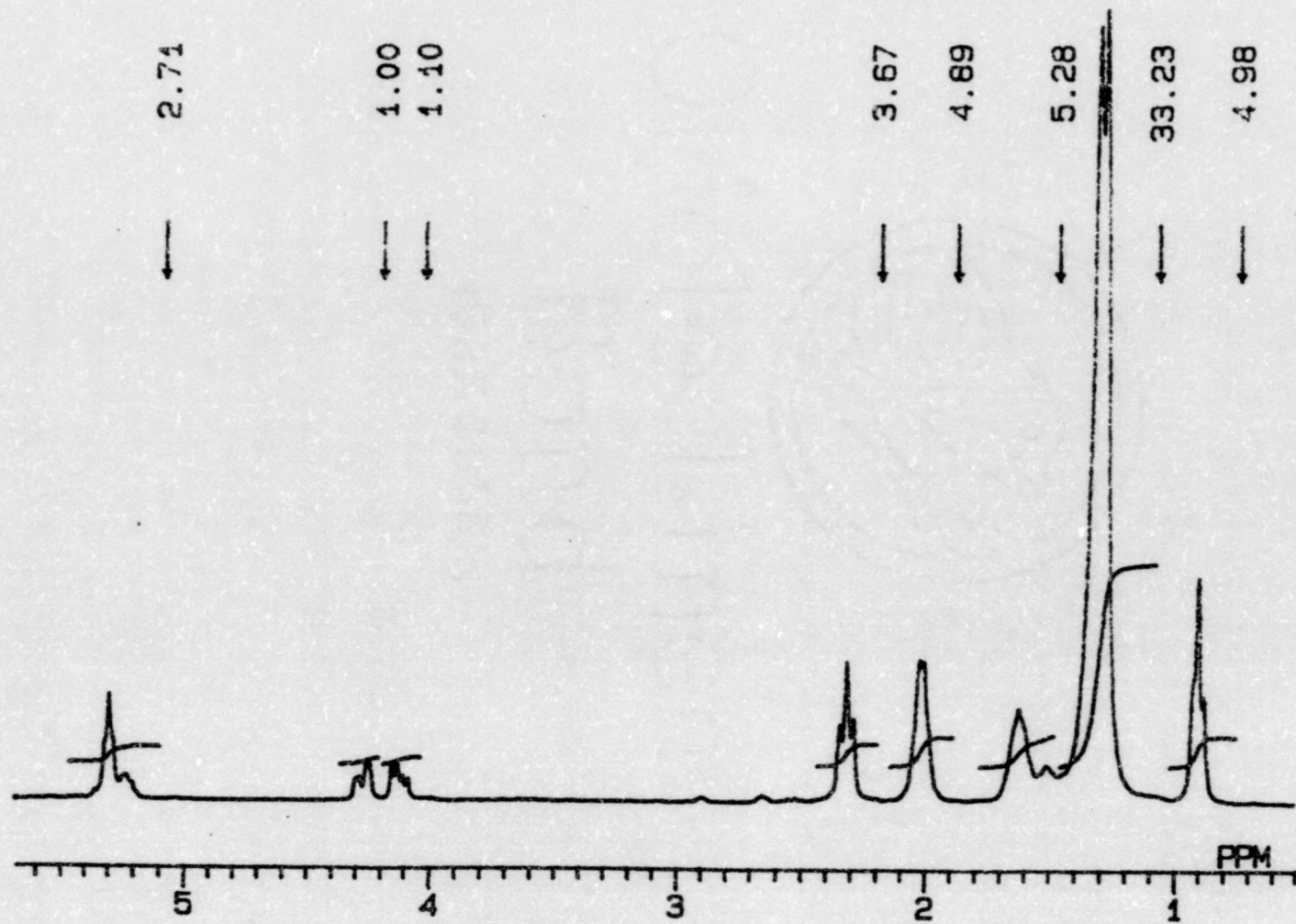


Figure 6. ^1H NMR spectrum of trioleoylglycerol heated at 160° in the presence of air for 10 hours.




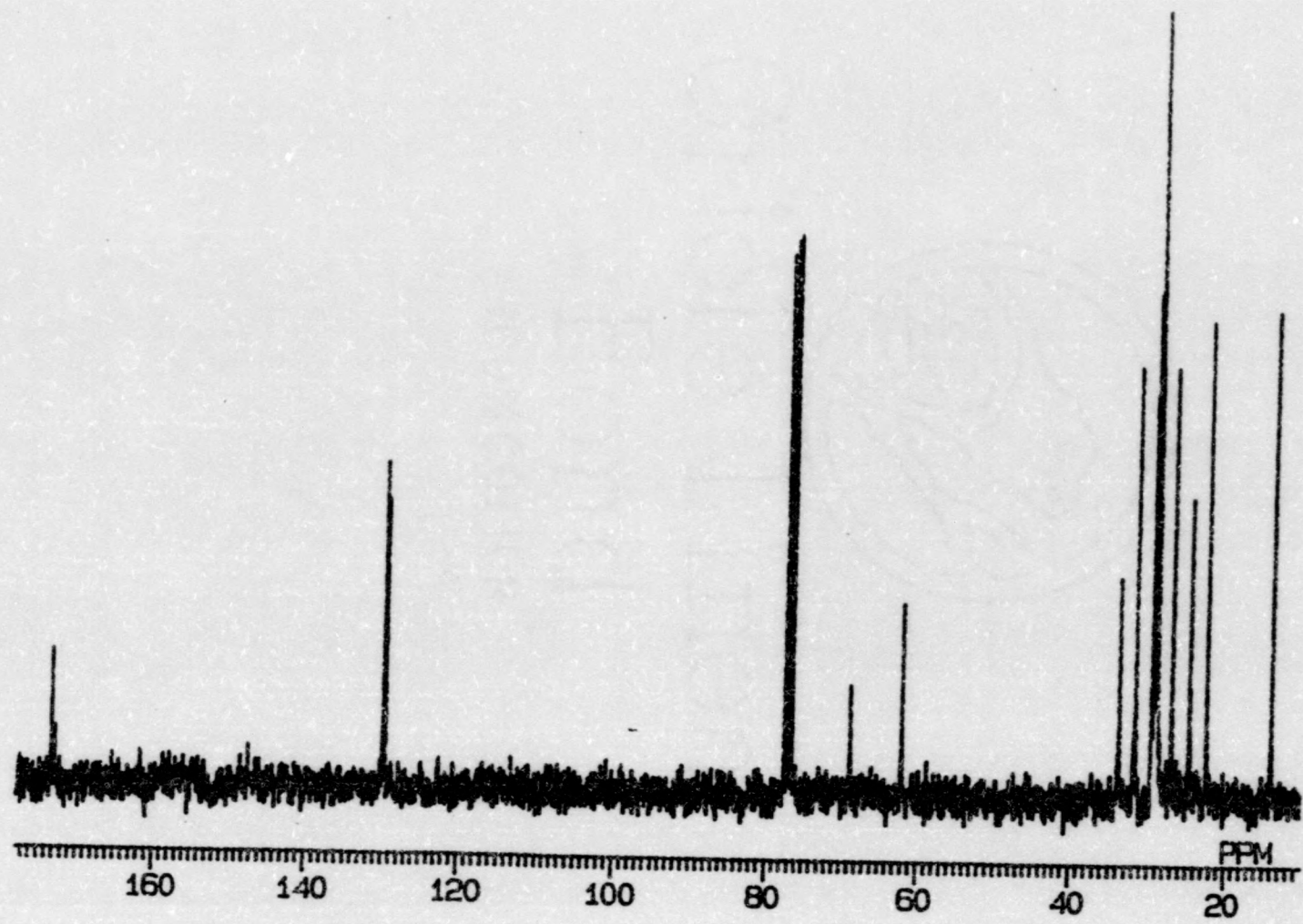


Figure 7. ^{13}C NMR spectrum of trioleoylglycerol heated at 160° in the presence of air for 10 hours.



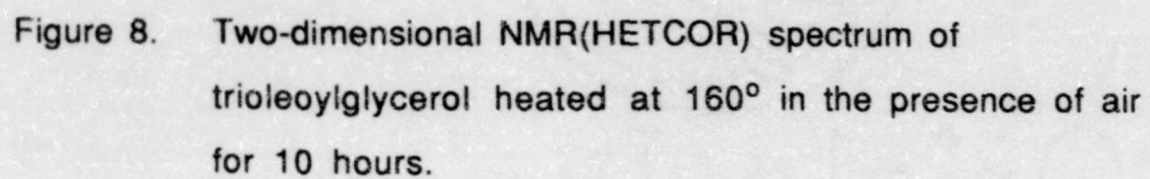


Figure 8. Two-dimensional NMR(HETCOR) spectrum of trioleoylglycerol heated at 160° in the presence of air for 10 hours.

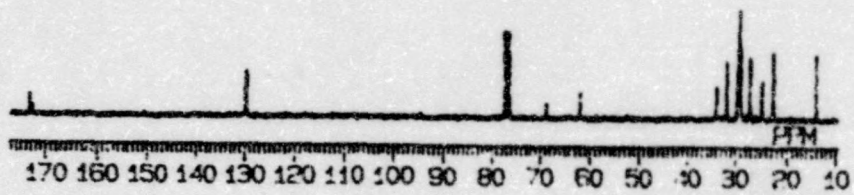
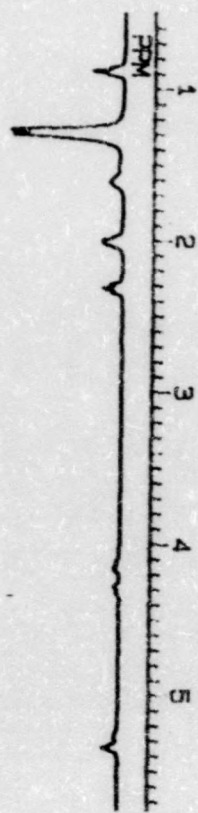


Figure 9. Two-dimensional NMR(COSY) spectrum of trioleoylglycerol heated at 160° in the presence of air for 10 hours.

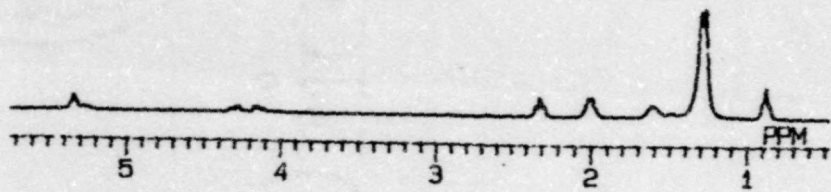
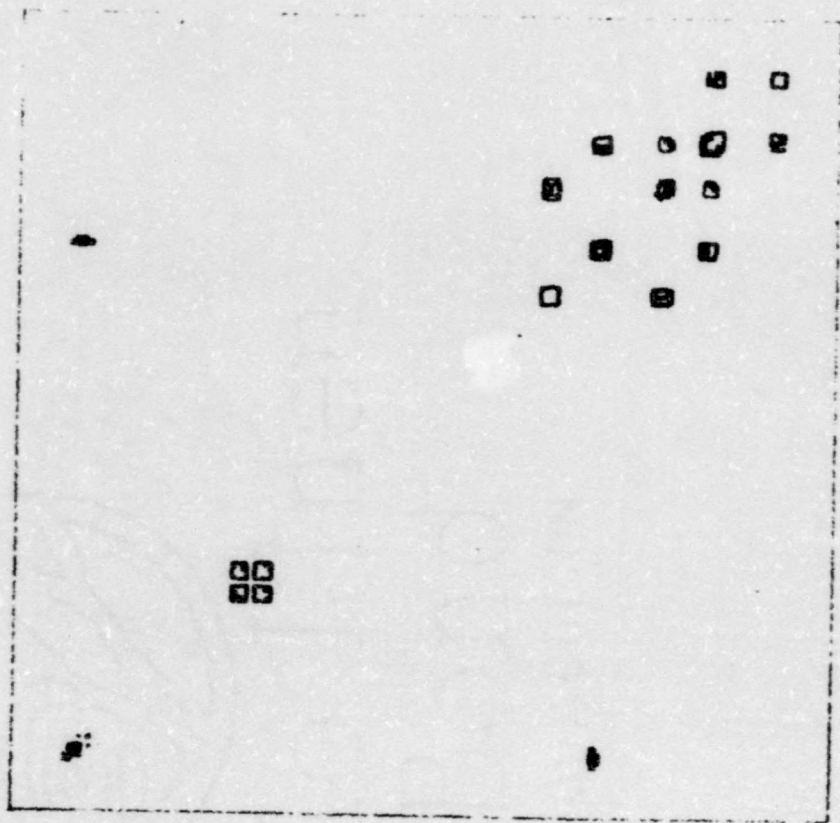
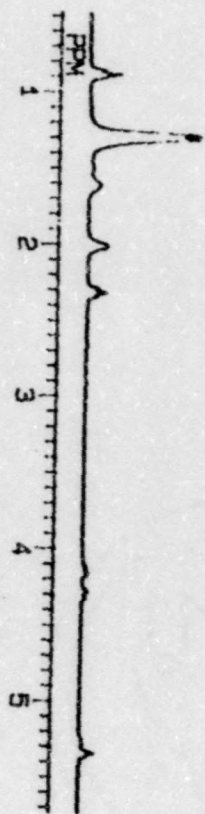
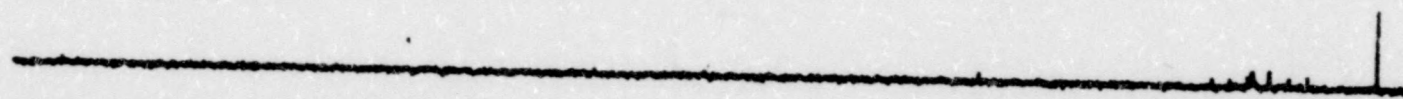


Figure 10. Two-dimensional NMR(DEPT) spectrum of trioleoylglycerol heated at 160° in the presence of air for 10 hours.

CH₃-GROUPS



CH₂-GROUPS



CH-GROUPS

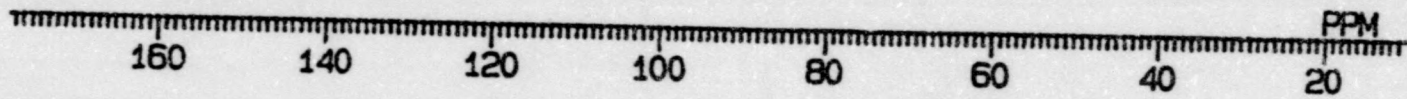
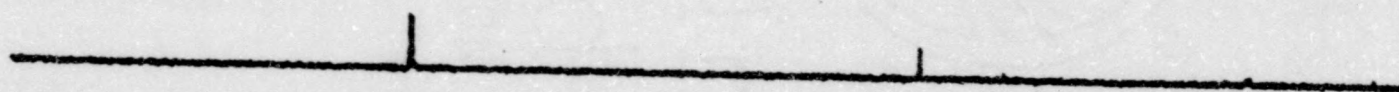


Figure 11. ^1H NMR spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.

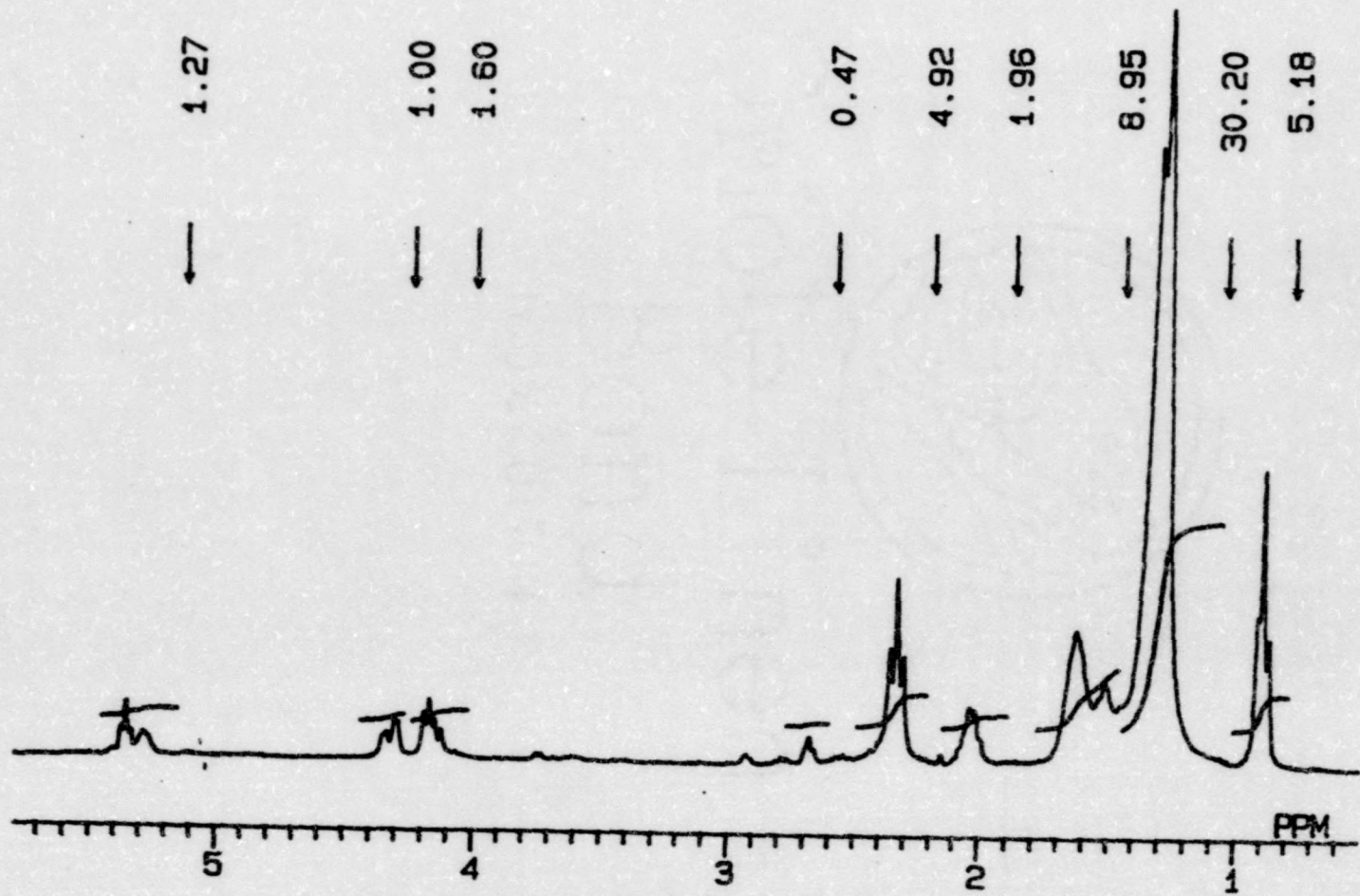


Figure 12. ^{13}C NMR spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.

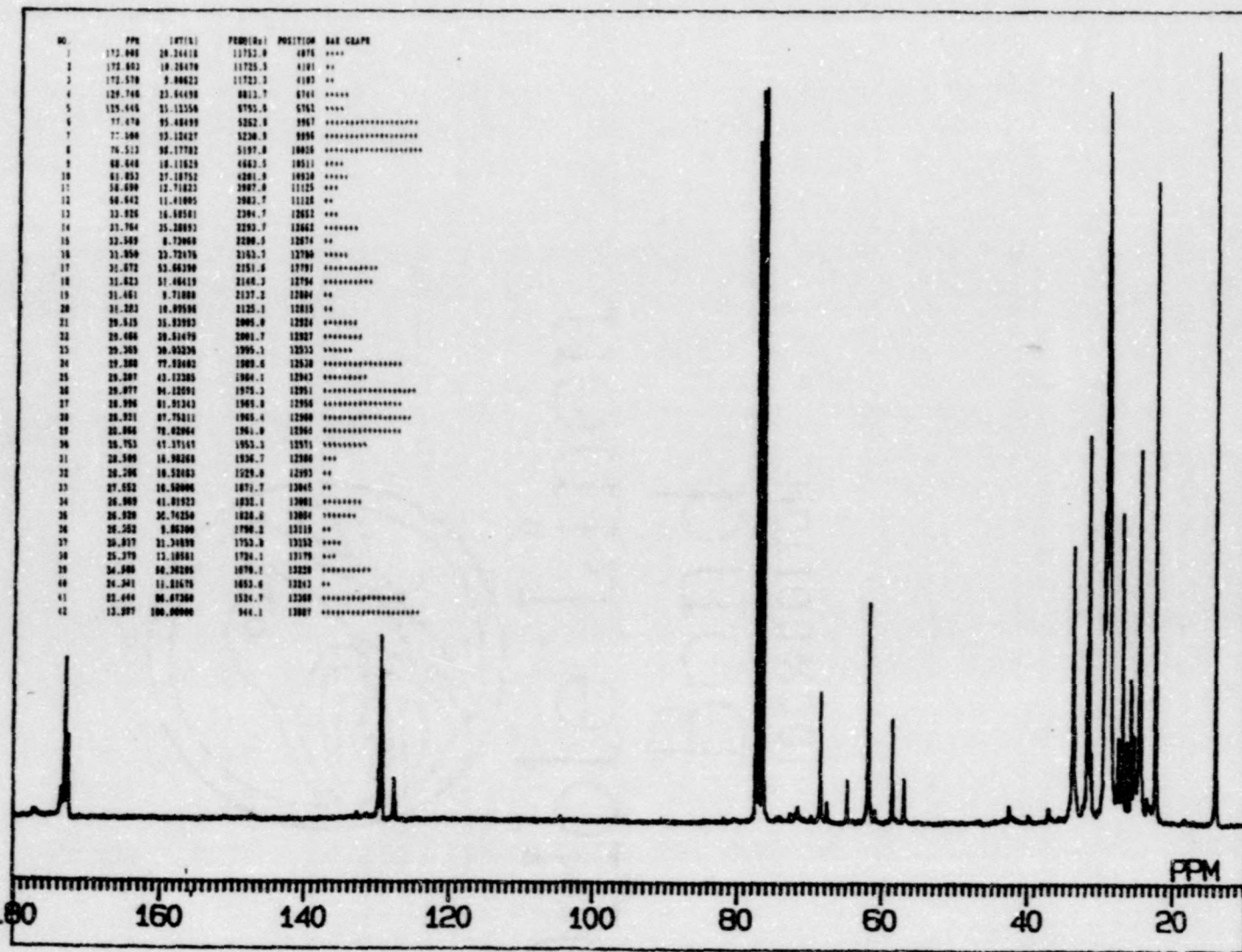
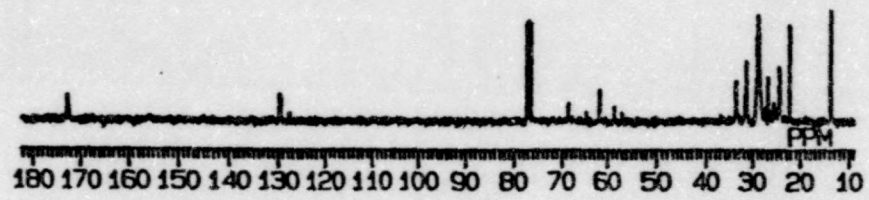
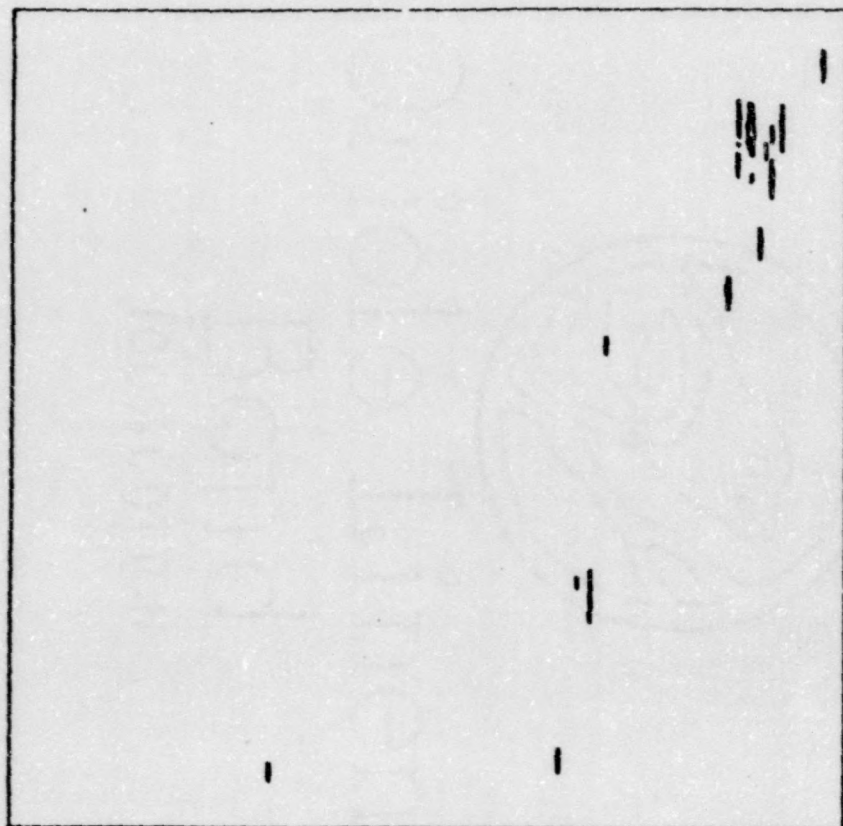
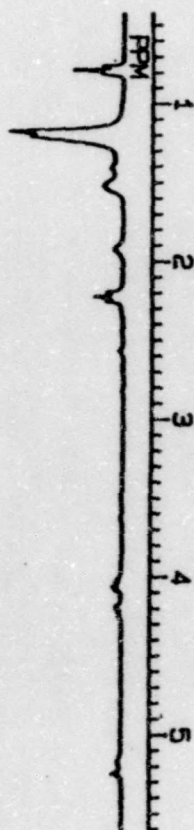


Figure 13. Two-dimensional NMR(HETCOR) spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.






Figure 14. Two-dimensional NMR(COSY) spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.

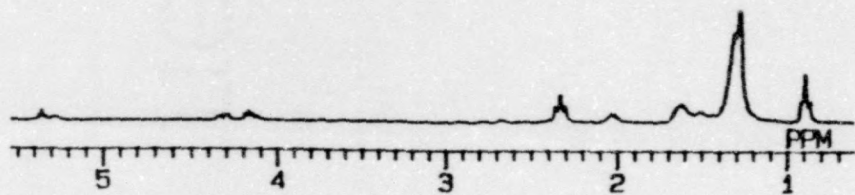
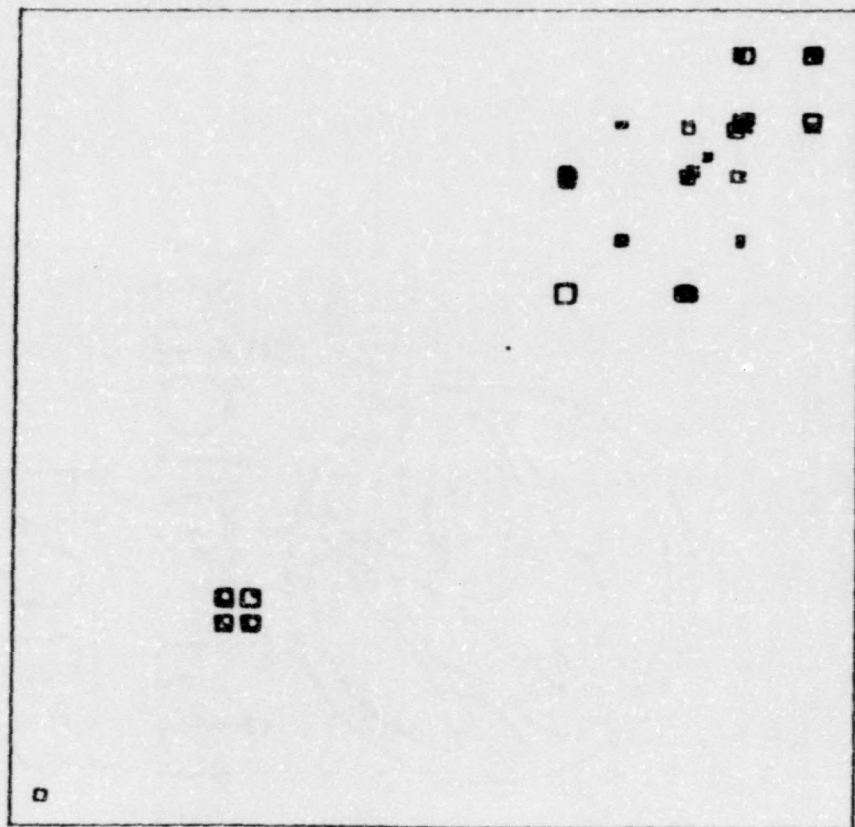
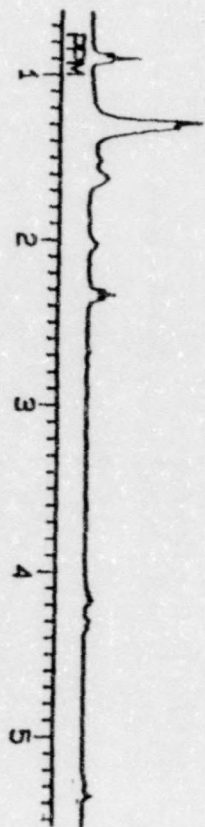
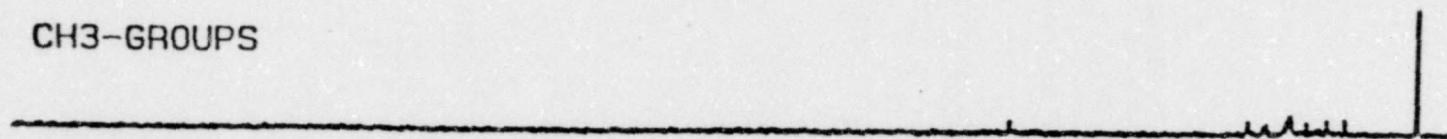
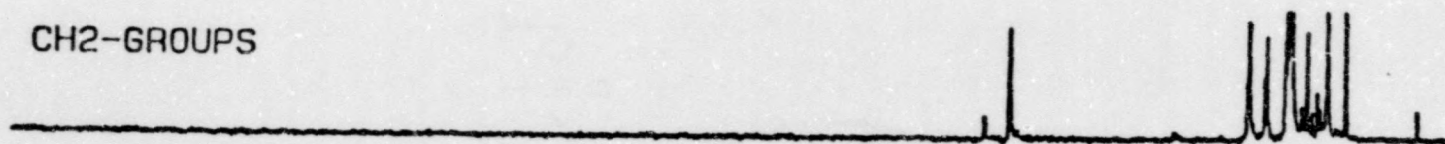


Figure 15. Two-dimensional NMR(DEPT) spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.

CH3-GROUPS



CH2-GROUPS



CH-GROUPS

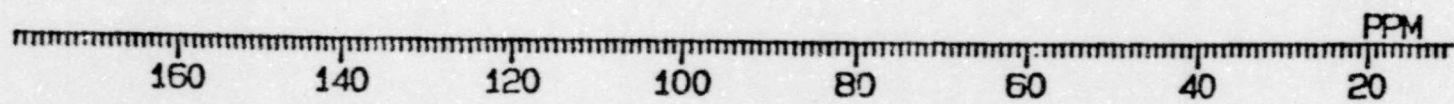
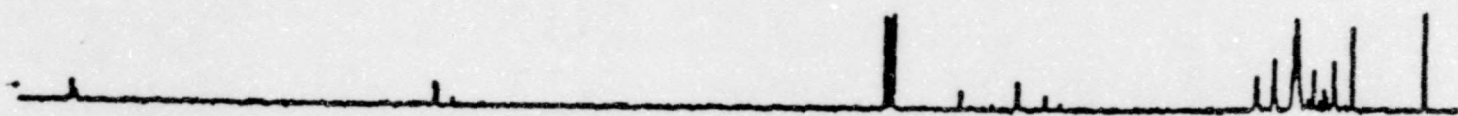
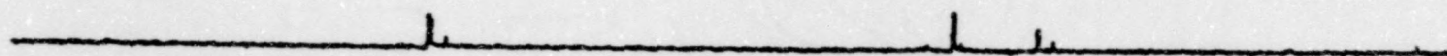


Figure 16. FT-IR spectrum of trioleoylglycerol.

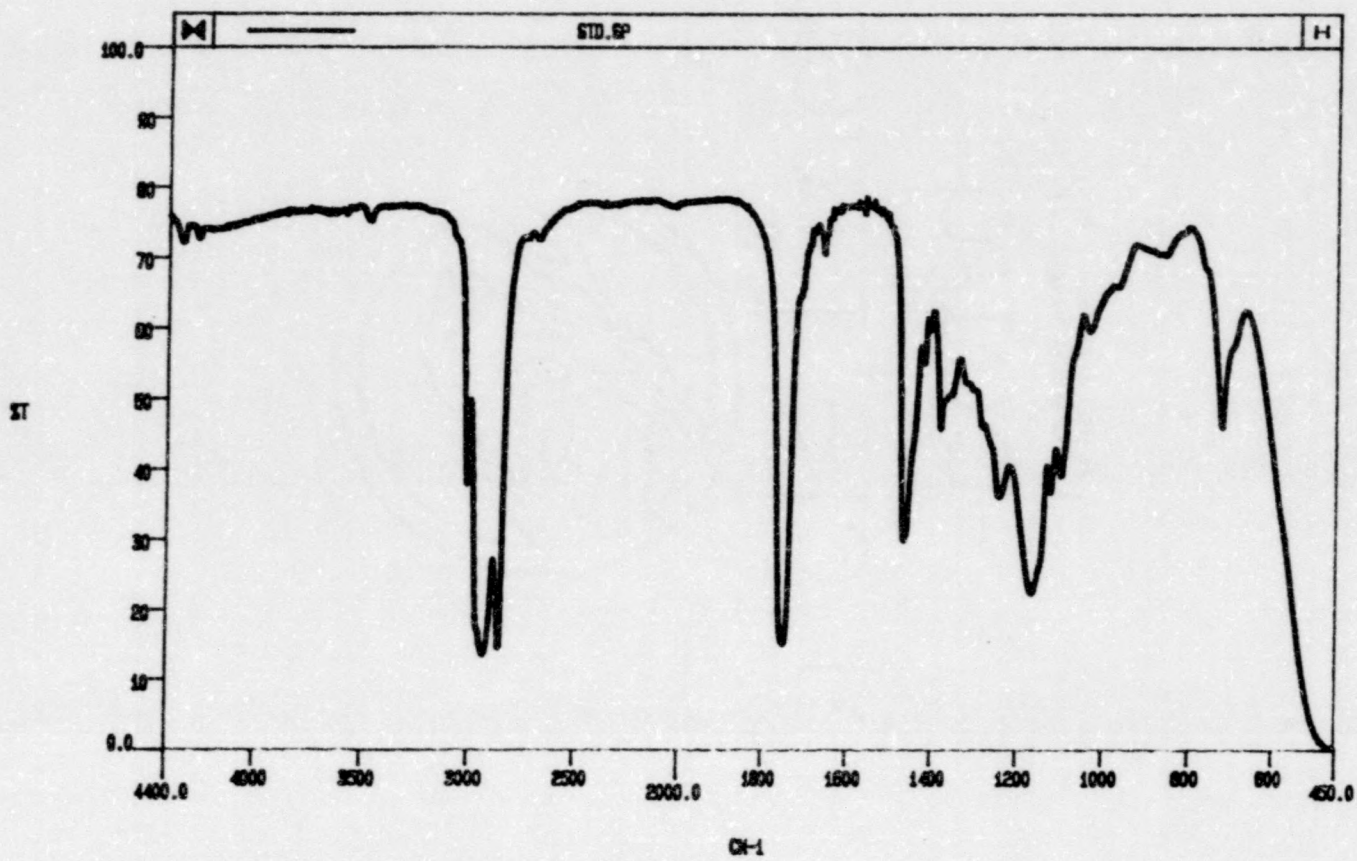
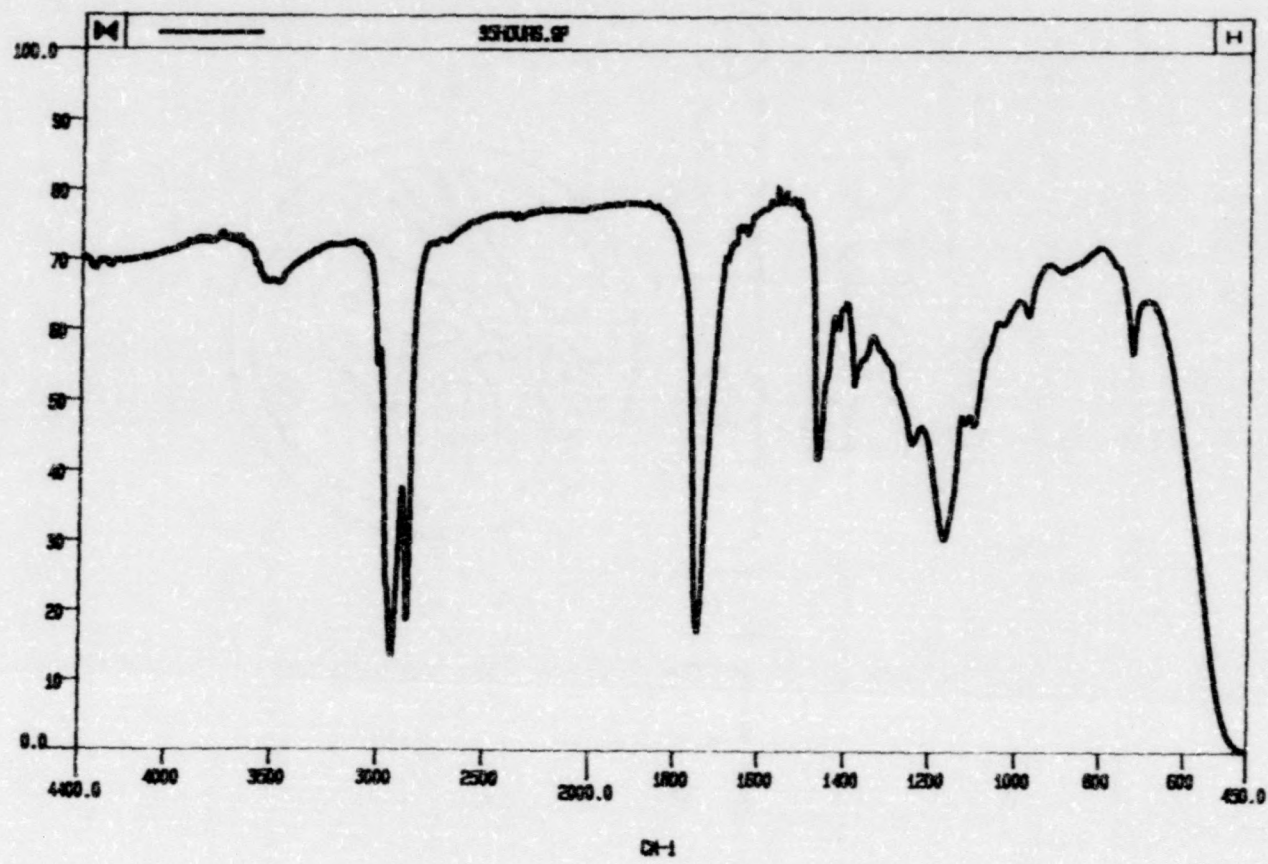


Figure 17. FT-IR spectrum of trioleoylglycerol heated at 160° in the presence of air for 35 hours.



CONCLUSIONS

Lipid oxidation easily occurs at temperatures used for frying foods in the presence of air and produces a multiplicity of compounds. The major primary product of trilinoleoylglycerol (3 double bonds) autoxidation was found to be hydroperoxide [11]. The oxidation conditions were mild (40°C), but the use of pure oxygen produced conjugated double bonds and hydroperoxide in measurable concentration.

The study of fat oxidation was conducted with trioleylglycerol (1 double bond) and used simulated frying conditions 160°C and air. The temperature was more severe, but pure oxygen and multiple double bonds were not present. The products of oxidation were found to be hydroperoxide and peroxide or epoxide shown by Frankel [12]. The results of this study indicate the presence of the same substances. The application of FT-NMR to the determination of oxidized oil yielded information indicating the spectra of the sample at 4 hours were not different from those of zero time, the spectra at 50, 70, 100 hours were not much different from those at 35 hours.

The effect of time on peroxide formation and decomposition shows four phases [13]: (1) induction period or free radical initiation phase; (2) free radical propagation, peroxide formation phase; (3) free radical termination phase, peroxide stabilization; and (4) peroxide decomposition phase. The process occurs at

different rates depending on the total unsaturation of the fat and many other associated factors.

Fats appear to go through a free radical initiation period during oxidation, which found to last between four and 10 hours. Oxidized reactions then move into a free radical propagation or peroxide formation phase, which started at about 10 hours and persisted for over 100 hours in the samples tested. The rate of formation then slows and a free radical termination phase is entered. In this phase peroxides and hydroperoxides are stabilized which, after a time period, begin to decompose.

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