The Mechanism of Low Levels of Nitrogen Dioxide Reaction With Unsaturated Fatty Acid Esters.

Aris Ann Gallon

Louisiana State University and Agricultural & Mechanical College

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The mechanism of low levels of nitrogen dioxide reaction with unsaturated fatty acid esters

Gallon, Aris Ann, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1990
The Mechanism of Low Levels of Nitrogen Dioxide Reaction with Unsaturated Fatty Acid Esters

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by

Aris Ann Gallon
B.S., Louisiana State University, 1984
December 1990
This work is dedicated to Jesus Christ.

I would like to thank:
Ph.D. Pryor and Ph.D. Church for their support and guidance.
My mother Evangeline Gallon and Father Solomon Gallon for their encouragement.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>List of Schemes</td>
<td>xiii</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xv</td>
</tr>
<tr>
<td>Abstract</td>
<td>xvi</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Chapter 1: The Reaction of Nitrogen Dioxide with Methyl Oleate.</td>
<td></td>
</tr>
<tr>
<td>a. Introduction</td>
<td>16</td>
</tr>
<tr>
<td>b. Experimental</td>
<td>18</td>
</tr>
<tr>
<td>c. Results and Discussion</td>
<td>25</td>
</tr>
<tr>
<td>III. Chapter 2: The Mechanism of Nitrogen Dioxide Initiated Autoxidation of Methyl Linoleate.</td>
<td></td>
</tr>
<tr>
<td>a. Introduction</td>
<td>29</td>
</tr>
<tr>
<td>b. Experimental</td>
<td>30</td>
</tr>
<tr>
<td>c. Results</td>
<td>50</td>
</tr>
<tr>
<td>d. Discussion</td>
<td>72</td>
</tr>
</tbody>
</table>

iii
Table of Contents (Continued)

IV. Chapter 3: Characterization of Allylic Nitrite(Nitro) Derivatives of Methyl Linoleate.
   a. Introduction.............................. 86
   b. Experimental............................... 87
   c. Results.................................... 91
   d. Discussion and Conclusion................. 109

V. Chapter 4: Reaction of Nitrogen Dioxide with a 50:50 Molar Methyl Oleate and Methyl Linoleate Solution.
   a. Introduction.............................. 115
   b. Experimental............................... 115
   c. Results.................................... 117
   d. Discussion ................................ 119

VI. Chapter 5: Nitrogen Dioxide Reaction with Methyl Linolenate.
   a. Introduction.............................. 121
   b. Experimental............................... 121
   c. Results.................................... 129
   d. Discussion................................ 148
Table of Contents (Continued)

VII. Chapter 6: Nitrogen Dioxide Catalyzed Isomerization of Methyl Linoleate and Methyl Linolenate Hydroperoxides.
   a. Introduction............................. 152
   b. Experimental............................. 153
   c. Results.................................. 158
   d. Discussion............................... 162

VIII. Chapter 7: The Preventative Antioxidant Abilities of Vitamin E and Vitamin C.
   a. Introduction............................. 165
   b. Experimental............................. 168
   c. Results................................... 171
   d. Discussion............................... 174

IX. Chapter 8: Conclusion.......................... 177

X. References.................................... 181

XI. Vita.......................................... 198
LIST OF TABLES

1. Table I: Proton NMR Data of the Vinyl Nitro Compound. 39
2. Table II: Proton NMR Data of the Vinyl Nitrate Compound. 42
3. Table III: Mole Percentage of Products Formed from Nitrogen Dioxide and Methyl Linoleate Reactions in Helium at 37°C. 66
4. Table IV: Mole Percentage of Products Formed from 50:50 Methyl Palmitate/Methyl Linoleate and Nitrogen Dioxide Reactions in Helium at 37°C. 67
5. Table V: Mole Percentage of Products Formed from Aqueous 50:50 Methyl Palmitate/Methyl Linoleate and Nitrogen Dioxide Reactions in Helium at 37°C. 68
6. Table VI: Mole Percentage of Products Formed from Methyl Linoleate and Nitrogen Dioxide Reactions in Air at 37°C. 69
7. Table VII: Mole Percentage of Products Formed from 50:50 Methyl Palmitate/Methyl Linoleate and Nitrogen Dioxide Reactions in Air at 37°C. 70
List of Tables (Continued)

8. Table VIII: Mole Percentage of Products Formed from Aqueous 50:50 Methyl Palmitate/Methyl Linoleate and Nitrogen Dioxide Reactions in Air at 37°C. 71

9. Table IX: NMR Data for the 13-cis,trans Isomers. 94

10. Table X: NMR Data for the 13-trans,trans Isomers. 95

11. Table XI: NMR Data of the 9-Allylic Nitrite(Nitro) Isomers. 96

12. Table XII: IR Stretching Frequencies cm⁻¹ of Nitro and Nitrite Compounds. 109

13. Table XIII: Mole Percentage of Products Formed from a 50:50 Methyl Oleate/Methyl Linoleate Reaction with 5 ppm of Nitrogen Dioxide (in Helium). 117

14. Table XIV: Hydroperoxide Fractions of Methyl Linolenate. 131

15. Table XV: Proton NMR Data of the Allylic Nitrite(Nitro) Isomers and Hydroperoxides of Methyl Linolenate. 132
List of Tables (Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.</td>
<td>Table XVI: Allylic Nitrite(Nitro) Isomers of Methyl Linolenate.</td>
<td>147</td>
</tr>
<tr>
<td>17.</td>
<td>Table XVII: Mole Percentage of Methyl Linolenate and Nitrogen Dioxide Products.</td>
<td>148</td>
</tr>
<tr>
<td>18.</td>
<td>Table XVIII: Proton NMR Data of Cyclic Peroxides.</td>
<td>156</td>
</tr>
<tr>
<td>19.</td>
<td>Table XIX: Hydroperoxide Isomers Reaction with Nitrogen Dioxide.</td>
<td>159</td>
</tr>
<tr>
<td>20.</td>
<td>Table XX: Moles of Products Formed from Nitrogen Dioxide Reacting with Methyl Linoleate in the Presence and Absence of Vitamin C and Vitamin E in Helium at 37°C.</td>
<td>172</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Figure 1: Phosphatidylcholine and Methyl Esters of Unsaturated Fatty Acids.</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Figure 2: Nitrogen Dioxide Filled Glass Bulb.</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Figure 3: The Bubbler Apparatus Used for the Nitrogen Dioxide Reactions.</td>
<td>23</td>
</tr>
<tr>
<td>4.</td>
<td>Figure 4: Gas Chromatogram and EI Spectra of Methyl Linoleate Isomers.</td>
<td>38</td>
</tr>
<tr>
<td>5.</td>
<td>Figure 5: Mole Percentage of Nitrogen Dioxide Disproportionating into Nitrite and Nitrate Anions.</td>
<td>55</td>
</tr>
<tr>
<td>6.</td>
<td>Figure 6: The Kinetic Chain Length of Nitrogen Dioxide and Methyl Linoleate Reactions in the Absence and Presence of Oxygen.</td>
<td>57</td>
</tr>
<tr>
<td>7.</td>
<td>Figure 7: Mole Percentage of Allylic Nitrite (Nitro) Isomers and Hydroperoxide Isomers Formed When Nitrogen Dioxide Reacts with Neat Methyl Linoleate in the Absence and Presence of Oxygen.</td>
<td>60</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

8. Figure 8: Mole Percentage of Allylic Nitrite (Nitro) Isomers and Hydroperoxide Isomers Formed When Nitrogen Dioxide Reacts with a 50:50 Methyl Linoleate/Methyl Palmitate Solution in the Absence and Presence of Oxygen.

9. Figure 9: Mole Percentage of Allylic Nitrite (Nitro) H-abstraction Products Formed as the Concentration of NO2 is Increased.

10. Figure 10: HPLC Chromatogram of the Allylic Nitrite(Nitro) Isomers and Hydroperoxide Isomers.

11. Figure 11: Gas Chromatograms of Methyl Linoleate Hydroperoxides and Allylic Nitrite (Nitro) Isomers.

12. Figure 12: Negative Methane Chemical Ionization Spectra of the 9-Hydroperoxide Isomers.

13. Figure 13: Negative Methane Chemical Ionization Spectra of the 13-Hydroperoxide Isomers.
**List of Figures (Continued)**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.</td>
<td>Figure 14: Negative Methane Chemical Ionization Spectra of the 9-Allylic Nitrite(Nitro) Isomers.</td>
<td>105</td>
</tr>
<tr>
<td>15.</td>
<td>Figure 15: Negative Methane Chemical Ionization Spectra of the 13-Allylic Nitrite(Nitro) Isomers.</td>
<td>106</td>
</tr>
<tr>
<td>16.</td>
<td>Figure 16: HPLC Chromatogram of the Starting Material and Reaction Mixture of a 50:50 Methyl Oleate/Methyl Linoleate and Nitrogen Dioxide Reaction Mixture.</td>
<td>118</td>
</tr>
<tr>
<td>17.</td>
<td>Figure 17: HPLC Chromatograms of Linolenate Hydroperoxides and Sodium Borohydride Reduced Linolenate Hydroperoxides.</td>
<td>125</td>
</tr>
<tr>
<td>18.</td>
<td>Figure 18: HPLC Chromatograms of Methyl Linolenate Allylic Nitrite(Nitro) Isomers and Methyl Linolenate Hydroperoxides.</td>
<td>130</td>
</tr>
<tr>
<td>19.</td>
<td>Figure 19: Gas Chromatograms of Methyl Linolenate Hydroperoxides and Allylic Nitrite(Nitro) Isomers.</td>
<td>133</td>
</tr>
<tr>
<td>20.</td>
<td>Figure 20: Negative Chemical Ionization Spectrum of the Allylic Nitrite(Nitro) Isomer Fraction I.</td>
<td>140</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.</td>
<td>Figure 21: Negative Chemical Ionization Spectrum of the Allylic Nitrite(Nitro) Isomer Fraction II.</td>
<td>141</td>
</tr>
<tr>
<td>22.</td>
<td>Figure 22: Negative Chemical Ionization Spectrum of the Allylic Nitrite(Nitro) Isomer Fraction III.</td>
<td>142</td>
</tr>
<tr>
<td>23.</td>
<td>Figure 23: Negative Chemical Ionization Spectrum of the Allylic Nitrite(Nitro) Isomer Fraction IV.</td>
<td>143</td>
</tr>
<tr>
<td>24.</td>
<td>Figure 24: Gas Chromatograms of the Cyclic Peroxide and Methyl Linolenate Hydroperoxide Fractions I and II.</td>
<td>157</td>
</tr>
<tr>
<td>25.</td>
<td>Figure 25: HPLC Chromatograms of Methyl Linolenate Hydroperoxide Fractions II and IV Reaction with Nitrogen Dioxide in Air.</td>
<td>161</td>
</tr>
<tr>
<td>26.</td>
<td>Figure 26: Vitamin C and Vitamin E.</td>
<td>167</td>
</tr>
</tbody>
</table>
LIST OF SCHEMES

1. Scheme I: The Addition Mechanism 6
2. Scheme II: The H-abstraction Mechanism 7
4. Scheme IV: Mechanism of Cyclic Peroxide Formation. 13
5. Scheme V: Isomerization of the Allylic Nitrite(Nitro) Isomers. 63
6. Scheme VI: Isomerization of the Allylic Nitrite(Nitro) Isomers by an Addition-Elimination Mechanism. 64
7. Scheme VII: Methyl Linoleate and Nitrogen Dioxide H-abstraction Mechanism. 73
8. Scheme VIII: Methyl Linoleate and Nitrogen Dioxide Addition Mechanism. 74
9. Scheme IX: The Formation of 2,4-Decadienal from Thermal Homolysis of the 9-Hydroperoxide and 9-Allylic Nitrite Isomers of Methyl Linoleate. 98
List of Schemes (Continued)


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>Allylic Nitrite(Nitro)</td>
</tr>
<tr>
<td>c,t</td>
<td>cis,trans</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatogram</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography-Mass Spectrometry</td>
</tr>
<tr>
<td>HONO</td>
<td>Nitrous Acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatograph</td>
</tr>
<tr>
<td>KCL</td>
<td>Kinetic Chain Length</td>
</tr>
<tr>
<td>MP</td>
<td>Methyl Palmitate</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometer</td>
</tr>
<tr>
<td>NCI</td>
<td>Negative Chemical Ionization</td>
</tr>
<tr>
<td>ppm</td>
<td>Part Per Million</td>
</tr>
<tr>
<td>RH</td>
<td>Hydroperoxide</td>
</tr>
<tr>
<td>t,t</td>
<td>trans,trans</td>
</tr>
<tr>
<td>16:0</td>
<td>Methyl Palmitate</td>
</tr>
<tr>
<td>18:1</td>
<td>Methyl Oleate</td>
</tr>
<tr>
<td>18:2</td>
<td>Methyl Linoleate</td>
</tr>
<tr>
<td>18:3</td>
<td>Methyl Linolenate</td>
</tr>
</tbody>
</table>
ABSTRACT

Nitrogen Dioxide is a toxic air pollutant that exists at less than 0.5 ppm in the atmosphere. This toxic compound is known to initiate autoxidation of unsaturated fatty acids both in vivo and in vitro. When autoxidation occurs in vivo, membrane damage that can lead to cell death can occur.

Low concentrations of nitrogen dioxide were shown to react with the polyunsaturated fatty acid esters, methyl linoleate and methyl linolenate, by a H-abstraction mechanism. However, methyl oleate, an unsaturated fatty acid ester, was demonstrated to react with a low concentration of nitrogen dioxide by only an addition mechanism. Although methyl oleate reacts by an addition mechanism, a 50:50 molar solution of methyl oleate and methyl linoleate reacted with a low level of nitrogen dioxide exclusively by a H-abstraction mechanism. Therefore, low levels of nitrogen dioxide will probably react with polyunsaturated fatty acid components of pulmonary lipids by a H-abstraction mechanism forming nitrous acid directly in the cell membrane.

Vitamin E was demonstrated to be able to act as a preventative antioxidant in the nitrogen dioxide and...
methyl linoleate reactions; but vitamin C could not prevent nitrogen dioxide from reacting with methyl linoleate by a H-abstraction mechanism. These results suggest that low levels of nitrogen dioxide will react with polyunsaturated fatty acids by a H-abstraction mechanism and that vitamin E can be used to prevent the reaction from occurring.
INTRODUCTION

Nitrogen dioxide is a toxic air pollutant that exists at less than 0.5 ppm in the atmosphere (1,2). Paramagnetic and brown in color, nitrogen dioxide coexists with its dimer dinitrogen tetroxide, a diamagnetic and colorless compound, at an equilibrium constant of $10^{-4}$M (2,3).

$$K_{eq} = 1 \times 10^{-4} \text{ M} = [\text{NO}_2]^2/\text{[N}_2\text{O}_4]$$

Although nitrogen dioxide is in equilibrium with its dimer dinitrogen tetroxide, at low concentrations of nitrogen dioxide the equilibrium favors nitrogen dioxide over dinitrogen tetroxide. Therefore, nitrogen dioxide is believed to be the reacting component at low concentrations of nitrogen dioxide (19).

In urban atmospheres, nitrogen dioxide is formed from the oxidation of nitrogen containing compounds by thermal combustion forming nitric oxide (NO) that reacts instantly with oxygen to form nitrogen dioxide (2).

$$2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$$
A mixture of nitric oxide and nitrogen dioxide or NOx is also found in cigarette smoke at a concentration in undiluted smoke between 150-650 ppm with negligible differences between filtered or unfiltered brands (2). Deep inhalation of the cigarette smoke allows about 95% of the NOx to remain in the lung (2). Cigars and pipes produce up to 1200 ppm of NOx (2).

Inhalation of nitrogen dioxide is known to cause inflammation in the lung, pulmonary edema, pulmonary fibrosis, bronchitis and has recently been linked to the spread of cancer (1, 2, 4-7). Nitrogen dioxide is believed to cause cancer to spread by reducing the number of immune cells in animals exposed to the toxic compound (4-7).

Studies have shown that nitrogen dioxide can cause damage to cell membranes that can eventually lead to cell death (8-13). This cell membrane damage is believed to be caused by autoxidation of unsaturated fatty acid components of phospholipids that comprise the membrane (8-13). When autoxidation of unsaturated fatty acids occur in the membrane hydroperoxides are formed. One of the overall results of hydroperoxide formation in the membrane is a decrease in the fluidity of the membrane which causes
a reduction in the transport through the membrane (9,14).

One of the pulmonary lipids found to increase in concentration in the lungs of rats upon exposure to 20-40 ppm of nitrogen dioxide is phosphatidylcholine (43,44) (Figure 1). Some of the pulmonary fatty acids comprising phosphatidylcholine are palmitic acid, oleic acid, linoleic acid, and arachadonic acid (43,44) (Figure I). When rats inhale 20-40 ppm of nitrogen dioxide an increase in palmitic acid incorporation in phosphatidylcholine accompanied with an increase in linoleic acid and arachadonic acid and a decrease in oleic acid components are detected(43). The increase in the fatty acids of pulmonary phosphatidylcholine is believed to result from the stimulation of net cellular synthesis (43). Since oleic acid, linoleic acid and arachadonic acid appear to be affected by nitrogen dioxide, the mechanism of environmentally relevant concentrations of nitrogen dioxide reaction with the methyl esters of oleic acid, linoleic acid, and linolenic acid (Figure 1) (a fatty acid used to synthesize arachadonic acid in the body) to initiate autoxidation was studied. Methyl linolenate was used as a model for arachadonic acid because linolenate is simpler and inexpensive to use.
Figure 1: Phosphatidylcholine and methyl esters of unsaturated fatty acids.
The only major difference between the two polyunsaturated fatty acids is that arachidonic acid is more oxidizable than linolenic acid.

Although low levels of nitrogen dioxide can initiate autoxidation of unsaturated fatty acids both in vivo (12,15,16) and in vitro (17,18), the mechanism of low levels of nitrogen dioxide initiated autoxidation of unsaturated fatty acids has not been confirmed as being one of H-atom abstraction instead of addition.

An earlier study by Ligthsey (19) demonstrated, by a product analysis, that the mechanism of nitrogen dioxide reaction with cyclohexene to initiate autoxidation changed from an addition mechanism at high concentrations of nitrogen dioxide to a H-atom abstraction mechanism at low concentrations of nitrogen dioxide. The same trend was proposed to occur with unsaturated fatty acids (19).

In the addition mechanism (19-22) (Scheme I), nitrogen dioxide can add to the double bond in a reversible step (23,24) to form a carbon centered radical (A). In the absence of oxygen, the carbon centered radical is trapped by another molecule of nitrogen dioxide forming a dinitro (B) or nitro-nitrite (C) compound. The nitro-compounds (B) and (C)
Scheme I

The Addition Mechanism

\[
\begin{align*}
\text{NO}_2 + \text{C} &= \text{C}^\cdot \rightleftharpoons \text{O}_2\text{N} - \text{C}^\cdot \text{C}^\cdot \\
&\quad \text{A}
\end{align*}
\]

\[
\begin{align*}
\text{A} + \text{NO}_2/\text{N}_2\text{O}_4 &\rightarrow \text{O}_2\text{N} - \text{C}^\cdot \text{C}^\cdot \text{NO}_2 + \text{O}_2\text{N} - \text{C}^\cdot \text{C}^\cdot \text{ONO} \\
&\quad \text{B} \quad \text{C}
\end{align*}
\]

\[
\begin{align*}
\text{H} \\
\text{O}_2\text{N} - \text{C}^\cdot \text{C}^\cdot \text{NO}_2 &\rightarrow \text{O}_2\text{N} - \text{C} = \text{C}^\cdot + \text{HONO} \\
&\quad \text{D}
\end{align*}
\]

\[
\begin{align*}
\text{D} + \text{C} &= \text{C} - \text{CH}_2 &\rightarrow &\text{O}_2\text{N} - \text{C}^\cdot \text{C} - \text{OO}^\cdot \\
&\quad \text{E} \\
\text{E} + \text{O}_2 &\rightarrow &\text{C} &= \text{C} - \text{CH} - \text{OO}^\cdot \\
&\quad \text{F}
\end{align*}
\]
Scheme II

The H-Abstraction Mechanism

\[ \text{NO}_2 + \text{C} = \text{C} - \text{CH}_2 \rightarrow \text{HONO} + \text{C} = \text{C} - \text{C} \]

\[ \text{E} + \text{NO}_2/\text{N}_2\text{O}_4 \rightarrow \text{C} = \text{C} - \text{CH}_2 - \text{NO}_2 + \text{C} = \text{C} - \text{CH}_2 - \text{ONO} \]

\[ \text{C} = \text{C} - \text{C} \quad + \quad \text{O}_2 \quad \rightarrow \quad \text{C} = \text{C} - \text{CH}_2 - \text{OO}^* \]

\[ \text{F} + \text{C} = \text{C} - \text{CH}_2 \rightarrow \text{C} = \text{C} - \text{CH}_2 - \text{OOH} + \text{C} = \text{C} - \text{C} \]
can eliminate nitrous acid to form vinyl nitro and
vinyl nitrite compounds. In the presence of oxygen,
the carbon centered radical (A) can react with a
molecule of oxygen forming a nitro-peroxyl radical
(D). The nitro-peroxyl radical is the species that
initiates autoxidation in the addition mechanism (19).
The nitro-peroxyl radical can abstract a hydrogen atom
from the allylic position of an alkene to form a
nitro-hydroperoxide and a resonance stabilized radical
(E). The resonance stabilized radical (E) can react
with oxygen to form an allylic peroxyl radical (F)
that can propagate the chain reaction.

At environmentally relevant concentrations of
nitrogen dioxide, the H-atom abstraction mechanism is
favored. In the H-atom abstraction mechanism (19)
(Scheme II), nitrogen dioxide can initiate
autoxidation by abstracting a hydrogen atom from the
allylic position of an alkene to form a resonance
stabilized radical (E) and nitrous acid. In the
absence of oxygen, nitrogen dioxide can combine with
the resonance stabilized radical (E), in a termination
step, to form an allylic nitro or nitrite compound.
In the presence of oxygen, the resonance stabilized
radical (E) can react with oxygen to form an allylic
peroxyl radical (F) that can propagate the chain
reaction.

The unsaturated fatty acid esters methyl oleate, methyl linoleate and methyl linolenate were shown indirectly to react by a hydrogen abstraction mechanism by (19):

a. a water analysis technique that monitored the amount of water produced from the decomposition of nitrous acid that was formed from nitrogen dioxide reacting by a H-atom abstraction mechanism in the absence of oxygen (19).

b. elemental analysis for nitrogen bound carbon when nitrogen dioxide in air was reacted with the esters. When nitrogen dioxide is reacted with unsaturated fatty acid esters in air, nitrogen bound carbon should only be formed if nitrogen dioxide reacts by an addition mechanism (19).

If nitrogen dioxide reacts with unsaturated fatty acids by a H-atom abstraction mechanism, nitrous acid can be formed directly in the cell membrane (17,19). The formation of nitrous acid may cause the nitrosation of amide linkages forming potent carcinogenic nitrosamides (25,26) in the cell membrane. A study by Lightsey demonstrated that nitrosamines can be formed when nitrogen dioxide
reacts with unsaturated fatty acids in the presence of amines (17). Another study by Kosaka et al., reported that nitrosamines are formed in animals when animals are exposed to nitrogen dioxide (27).

However, a product study by Kobayashi of the reaction of methyl oleate with 5070 ppm of nitrogen dioxide demonstrated that addition was the only mechanism occurring (28). This study did not disprove Ligthsey's work because the concentration of nitrogen dioxide used for the product study was one in which the addition mechanism should predominate over the H-atom abstraction mechanism (19).

In the study reported by Kikugawa (29), nitrogen dioxide (100 ppm) was reacted with methyl linoleate and methyl linolenate. A product study of the nitrogen dioxide in air reaction with polyunsaturated fatty acids demonstrated that nitrogen dioxide could initiate autoxidation (29). However, their results were inconclusive as to whether nitrogen dioxide initiated the reaction by a H-abstraction mechanism or an addition mechanism (29). In order to determine whether environmentally relevant concentrations of nitrogen dioxide can react with unsaturated fatty acids by a H-atom abstraction mechanism to initiate autoxidation, a product study of the reaction of
methyl oleate, methyl linoleate and methyl linolenate with a low level of nitrogen dioxide at 37°C in the absence of oxygen was performed. When nitrogen dioxide is reacted with unsaturated fatty acids in the absence of oxygen, only initiation and termination reactions can occur. The mechanism that predominates at a low concentration of nitrogen dioxide can be determined by identifying and quantifying the addition and H-abstraction products formed from the reaction. The methyl linoleate study is a complete study of the products formed from low levels of nitrogen dioxide reacting with methyl linoleate in the absence and presence of oxygen at 37°C using: neat methyl linoleate, a 50:50 molar solution of methyl palmitate and methyl linoleate, and a 50:50 molar methyl palmitate/methyl linoleate pH = 7.4, .5M buffered aqueous solution.

A study by Castle (30) demonstrated that nitrogen dioxide reacted with the O-H bond of hydroperoxides instead of the O-O bond. An earlier study demonstrated that hydrogen peroxide reacted with nitrogen dioxide in the gas phase by homolysis of the O-O bond (31). If low concentrations of nitrogen dioxide can react with hydroperoxides of polyunsaturated fatty acids by a H-atom abstraction
mechanism, the isomerization (32) and cyclic peroxide formation (33) of the hydroperoxides of polyunsaturated fatty acids would be catalyzed. The formation of cyclic peroxides is important because cyclic peroxides can decompose to malonaldehyde a compound that is known to cross-link proteins (34,35).

The hydroperoxides of linoleate were demonstrated by Porter et al. to isomerize by initially forming the peroxyl radical (32). Once the peroxyl radical is formed, oxygen can leave forming a resonance stabilized radical that can isomerize to another isomer and add another molecule of oxygen (Scheme III) (32). The hydroperoxide isomers isomerize by the following mechanism (32):

Scheme III

The Mechanism of Linoleate Hydroperoxide Isomerization
The linolenate hydroperoxides can not isomerize (36), but the 13-hydroperoxides and 12-hydroperoxides of linolenate can form cyclic peroxides when the peroxyl radical is formed (Scheme IV) (37). The outer 16-hydroperoxides and 9-hydroperoxides of linolenate can not form cyclic peroxides (36).

Scheme IV
Mechanism of Cyclic Peroxide Formation
In order to determine if nitrogen dioxide can catalyze the isomerization of methyl linoleate hydroperoxides and the cyclic peroxide formation of the methyl linolenate hydroperoxides by a H-atom abstraction mechanism, a low concentration of nitrogen dioxide was reacted with the hydroperoxides of methyl linoleate and the 12-hydroperoxides of methyl linolenate.

Vitamin E (alpha-tocopherol) and vitamin C (ascorbic acid) are known biological antioxidants (38,39). Vitamin E is a major lipid soluble antioxidant that is known to inhibit autoxidation of unsaturated fatty acids (38). Vitamin C is a water soluble antioxidant that can reduce tocopheroyl radicals to tocopherol and inhibit autoxidation of unsaturated fatty acids (40-42). Both vitamin E and vitamin C have been shown to inhibit autoxidation initiated by nitrogen dioxide (60,61). In order to determine whether vitamin E or vitamin C can also act as a preventative antioxidant, nitrogen dioxide in the absence of oxygen was reacted with methyl linoleate in the presence of vitamin E and vitamin C. If vitamin E or vitamin C can act as a preventative antioxidant, addition and H-abstraction termination products of unsaturated fatty acid esters should not be detected.
Presented is the mechanism of low concentrations of nitrogen dioxide reaction with unsaturated fatty acids at 37°C and the antioxidant than can be used to prevent the reaction from occurring. The study of whether nitrogen dioxide can catalyze the isomerization and cyclic peroxide formation of hydroperoxides of polyunsaturated fatty acids is also presented.
CHAPTER 1
The Reaction of Nitrogen Dioxide with Methyl Oleate

Nitrogen dioxide is known to initiate autoxidation of unsaturated fatty acid components of phospholipids that comprise cell membranes (43,44). Oleic acid which is an 18-carbon unsaturated fatty acid (Figure 1) is a constituent of phosphatidyl choline, a pulmonary phospholipid. An earlier product study by Kobayashi demonstrated that 5070 ppm of nitrogen dioxide reacted with oleic acid by an addition mechanism to initiate autoxidation (28). However, the mechanism at an environmentally relevant concentration of nitrogen dioxide has not been confirmed as being one of H-atom abstraction or addition.

A study by Lightsey(19) demonstrated, by elemental analysis for carbon-bound nitrogen in methyl oleate and nitrogen dioxide in air reaction mixtures, that the amount of nitrogen dioxide reacting by an addition mechanism decreased as the concentration of nitrogen dioxide decreased. The decrease in the amount of addition products detected by elemental analysis for carbon-bound nitrogen was assumed to be
caused by an increase in the amount of H-atom abstraction occurring as the concentration of nitrogen dioxide was decreased (19). Lightsey demonstrated that methyl oleate reacted by a H-abstraction mechanism by detecting the formation of water formed from a nitrogen dioxide and methyl oleate reaction in the absence of oxygen (19). The water detected in the reaction originated from the decomposition of nitrous acid (45,46). The nitrous acid was assumed to be formed from nitrogen dioxide abstracting a hydrogen atom from methyl oleate (19). Since nitrous acid can also be formed from the decomposition of dinitro and nitro-nitrite compounds to form vinyl nitro and vinyl nitrite products (Scheme I) (19), the water analysis technique may not be an accurate test for H-atom abstraction.

In order to determine whether a low concentration of nitrogen dioxide would react with methyl oleate by a H-atom abstraction mechanism, a product study of the reaction of methyl oleate with 5 ppm of nitrogen dioxide in the absence of oxygen was performed. When the reaction is performed in the absence of oxygen, only initiation and termination reactions can occur.

If nitrogen dioxide reacts with methyl oleate by an addition mechanism (19) (Scheme I), nitrogen
dioxide would add to the double bond in a reversible step forming a carbon centered radical (A). This carbon centered radical can terminate with another molecule of nitrogen dioxide forming a dinitro or nitro-nitrite compound that can eliminate nitrous acid to form a vinyl nitro or vinyl nitrite compound (Scheme I).

If nitrogen dioxide reacts with methyl oleate by a H-atom abstraction mechanism (19), a resonance stabilized radical would be formed (Scheme II). The resonance stabilized radical can terminate with another molecule of nitrogen dioxide to form an allylic nitro or nitrite compound.

The mechanism for the methyl oleate and nitrogen dioxide reaction that predominates at an environmentally relevant concentration of nitrogen dioxide was determined by identifying and quantifying the products detected (95).

**EXPERIMENTAL**

Chemicals:

Methyl oleate (99% by GC, Sigma) was purified from the hydroperoxide contaminants by passing methyl oleate in oxygen free hexane (HPLC grade, Mallinckrodt) through four pasteur pipette columns
containing 1.0 g of alumina (Aldrich, neutral). The last column contained 0.02 g of pentetic acid (DTPA) in the tip to remove any trace metals. The columns were rinsed with hexane (oxygen free) before adding the methyl oleate solution. The methyl oleate was concentrated by blowing a stream of nitrogen over the solution. The entire purification process was performed in a glovebag under an atmosphere of nitrogen.

Methyl elaidate (99 % by GC, Sigma) was used to determine the response factor and retention time for the methyl elaidate formed from the nitrogen dioxide and methyl oleate reaction.

Methyl linelaidate (99 % by GC, Sigma) was used as the internal standard for the methyl oleate and nitrogen dioxide reaction mixture.

d-Carvone (96 %, Aldrich) was used as an internal standard for HPLC analysis.

Nitrogen dioxide was generated from its dimer dinitrogen tetroxide (99.5 %, Matheson). Dinitrogen tetroxide and phosphorus pentoxide (97 %, Aldrich) was placed in a glass bulb (Figure 2) equipped with a teflon valve (47) and purged of oxygen with argon by freezing and thawing the dinitrogen tetroxide three times. After each freeze-thaw cycle, the pressure was
Figure 2: Nitrogen dioxide filled glass bulb.
released by slightly opening the teflon valve.

Hexane and isopropanol (HPLC grade, Mallinckrodt) were purged of oxygen and used for high performance liquid chromatography (HPLC).

Helium (99.9999 % chromatographic grade, Air Products) was used as the carrier gas for the reaction.

Nitrogen and Argon (99.996 + %, Liquid Carbonic) were used for the glove bag and to purge the nitrogen dioxide bulb.

A Thymol trap was made by dissolving 0.4 g of thymol (Sigma) and 4 g of sodium hydroxide (97 %, EM Science) in 1 liter of deionized water(47).

Naphthylethylenediamine solution was made by dissolving 0.05 g of naphthylethylenediamine (98 %, Sigma) in 100 ml of deionized water(47).

Sulfanilimide solution was made by dissolving 28 g of sulfanilimide (98%, Aldrich) in a solution of 200 mL of 85 % phosphoric acid (85 %, Mallinckrodt) and 200 ml of deionized water(47).

Saltzmann buffer was made by mixing equal parts of a 0.1 N sodium hydroxide solution with 42.5 % phosphoric acid(47).
Instrumentation:

A high performance liquid chromatograph (HPLC) Varian 5000 series with a UV detector (Vari-Chrom) set at a 215 nm wavelength with a 16 nm slit width was used to analyze the methyl oleate and nitrogen dioxide reaction mixture. A 25 X 0.46 cm silica column (Rainin) was used with a 99:1 hexane/isopropanol solvent system at a flowrate of 1 ml/min.

A Varian 3740 gas chromatograph equipped with a flame ionization detector was also used to analyze the methyl oleate and nitrogen dioxide reaction mixture. A 100 m X 0.25 mm SP-2560 (cyano-polysiloxane) fused silica capillary column at an isothermal temperature of 200°C was used for GC analysis. The injection port temperature was 250°C.

Methods:

The bubbler apparatus shown in figure 3 was assembled in the glovebag under an atmosphere of nitrogen. The nitrogen dioxide bulb was opened and equilibrated for one hour. The ultra pure helium carrier gas had a flow rate of 60 mL/min. The temperature of the constant temperature bath was 37°C. Nitrogen dioxide in a helium carrier gas was bubbled through a 100 mL thymol trap for one hour to determine
Figure 3: The bubbler apparatus used for the nitrogen dioxide reactions.
the nitrogen dioxide concentration in the carrier gas. A Saltzman analysis (48) was performed on 2 mL of the thymol trap solution. The Saltzman analysis entailed adding 2 mL of sulfanilimide solution and 0.3 mL of napthylethylenediamine solution to 2 mL of the thymol trap solution that was bubbled with nitrogen dioxide and to 2 mL of the thymol trap that was not bubbled with nitrogen dioxide for a reference (47). The reddish-violet color was developed over a 1 hr period and the solutions were diluted with the Saltzman buffer to 10 mL. The absorbance at a maximum wavelength of 540 nm [molar absorptivity 49,000 M\(^{-1}\)cm\(^{-1}\)] (47) and the efficiency of the trap (70 ± 4.0 %) (96) were used to determine the concentration of nitrogen dioxide in the trap solution. The concentration of nitrogen dioxide in the carrier gas was determine by the following equation:

\[
\text{ppm} = \frac{[\text{moles NO2}]}{[\text{moles of NO2} + \text{moles of He}]} \times 10^6
\]

After the 1 hr blank was run, 1.5 mmoles of methyl oleate (neat) was added to the bubbler. Nitrogen dioxide (5 ppm) in He was bubbled through the fatty acid ester for 1 hr. The 100 mL thymol trap used for the reaction was also analyzed by a Saltzman
analysis. The difference in the amount of nitrogen
dioxide in the trap used as a blank to determine the
nitrogen dioxide concentration in the carrier gas and
the trap used in the reaction was taken as the amount
of nitrogen dioxide incorporated in the methyl oleate.
The neat methyl oleate reaction mixture and methyl
oleate starting material were analyzed by HPLC and GC
analysis.

The reaction mixture (0.020 mL) and starting
material (0.020 mL) were both dissolved in 0.10 mL of
carvone solution \([d = 2.16 \times 10^{-4} \text{ g/ml in hexane}]\) and
analyzed by HPLC analysis. The reaction mixture
(0.010 mL) and starting material (0.010 mL) were each
dissolved in 1.0 mL of hexane and analyzed by GC
analysis. The formation of methyl elaidate was
confirmed by spiking the methyl oleate reaction
mixture with a known sample of methyl elaidate.
Methyl linelaidate \([1.2 \times 10^{-7} \text{ moles}],\) the internal
standard, was added to each methyl oleate solution and
the solutions were analyzed again by GC analysis.

RESULTS AND DISCUSSION

The HPLC analysis of the starting material and
reaction mixture demonstrated that no products were detected.
However, GC analysis of the reaction mixture demonstrated that methyl elaidate was formed. Methyl elaidate is an addition product originating from the addition of nitrogen dioxide to the double bond followed by an elimination of the nitrogen dioxide to form the trans methyl oleate (Scheme I). The amount of methyl elaidate formed accounts for 45% of the nitrogen dioxide used in the reaction. The other 45-55% of nitrogen dioxide used in the reaction is believed to have re-formed methyl oleate in the addition-elimination step. Methyl oleate is reformed because the moment of inertia for rotation of the central C-C bond of the intermediate carbon centered radical is the same for forming the trans and cis double bonds (24). The conversion of methyl oleate to methyl elaidate was 0.1 ± 0.01%. The product detected accounts for 95 ± 5% of the methyl oleate reacted. The values above are an average of four GC analysis.

Nitrogen dioxide (5 ppm) in the absence of oxygen reacts with methyl oleate exclusively by an addition mechanism instead of a H-atom abstraction mechanism. If the amount of H-abstraction that might occur is estimated from methyl oleates' $k_{\text{abstr.}}/H = 0.22 \text{ M}^{-1}\text{s}^{-1}$ (49) and methyl linoleates $k_{\text{abstr.}}/H = 31 \text{ M}^{-1}\text{s}^{-1}$ (49), the ratio of the rate constants is $k_{18:1}/k_{18:2} = 7.1 \times 10^{-3}$. 
When methyl linoleate (1.5 mmoles) is reacted with 6.8 ppm of nitrogen dioxide for one hour 3.2 X 10^{-6} moles of allylic nitrite(nitro) H-abstraction products are formed (Chapter 2). If the amount of H-abstraction products formed from the methyl linoleate and NO_{2} reaction is multiplied by the ratio of 18:1 and 18:2 abstractability rate constants, the moles of allylic nitrite(nitro) products formed from methyl oleate under similar conditions would be 2.3 X 10^{-8} moles. The moles of the methyl elaidate addition product formed from the 5 ppm of methyl oleate and nitrogen dioxide reaction is 4.5 X 10^{-8} moles. Therefore, the addition mechanism still predominates over the H-abstraction mechanism. The results suggest that the decrease in the amount of addition occurring in the methyl oleate study performed by Ligthsey was not caused by an increase in the amount of H-atom abstraction. The results above also prove that the water detected in the water analysis technique for the methyl oleate and nitrogen dioxide reaction mixture performed by Lightsey was not formed from nitrous acid produced from a H-atom abstraction mechanism. The water detected was probably formed from nitrous acid produced from the formation of vinyl nitro or vinyl nitrite compounds (Scheme I).
Hydrogen abstraction not occurring when a low concentration of nitrogen dioxide reacted with methyl oleate may be caused by the allylic hydrogens of methyl oleate \( k_{\text{abstr}/H} = 0.22 \text{ M}^{-1}\text{s}^{-1} \) \(^{(49)}\) not being as abstractable as cyclohexene's \( k_{\text{abstr}/H} = 1.5 \text{ M}^{-1}\text{s}^{-1} \) \(^{(49)}\). In order to determine if low concentrations of nitrogen dioxide can react with an unsaturated fatty acid by a H-atom abstraction mechanism, an unsaturated fatty acid with abstractable allylic hydrogens comparable to cyclohexene's must be studied. Methylinoleate with a \( k_{\text{abstr}} = 31 \text{ M}^{-1}\text{s}^{-1} \) \(^{(49)}\) was the next unsaturated fatty acid ester studied to determine if low concentrations of nitrogen dioxide could react by a H-atom abstraction mechanism.
Methyl linoleate is an eighteen carbon polyunsaturated fatty acid (Figure 1) that is a constituent of phosphatidyl choline, a pulmonary phospholipid (44). Nitrogen dioxide is known to initiate autoxidation of linoleate (29); however, the mechanism of initiation has not been confirmed as being one of H-abstraction instead of addition. A study by Kikugawa et al. (29) reported that nitrogen dioxide can initiate the autoxidation of linoleate but did not confirm that the mechanism of initiation was a H-abstraction mechanism instead of an addition mechanism.

A study performed by Lightsey (19) demonstrated through elemental analysis for carbon-bound nitrogen and a water analysis technique of nitrogen dioxide in air and nitrogen reactions with unsaturated fatty acids that unsaturated fatty acids should react with low concentrations of nitrogen dioxide by a H-atom abstraction mechanism (19) (Scheme II). However, methyl oleate was previously shown to react with 5 ppm
of nitrogen dioxide in the absence of oxygen exclusively by an addition mechanism. In order to determine whether methyl linoleate can react with environmentally relevant concentrations of nitrogen dioxide by a H-abstraction mechanism, low concentrations of nitrogen dioxide were reacted with methyl linoleate under various conditions.

EXPERIMENTAL

Chemicals:

Methyl linoleate (99% by GC, Sigma) was purified by passing methyl linoleate in hexane (oxygen free) through four columns containing 1.0 g of alumina (neutral, Aldrich). The last column had 0.02 g of pentetic acid (DTPA) in the tip. The purification was done in a glove bag under an atmosphere of nitrogen.

Methyl palmitate (99%, Sigma) was used without purification.

The remainder of the chemicals used in the study are described in chapter 1.

Instrumentation:

The UV spectra were taken on a Hewlett Packard 8451A diode array spectrophotometer and the IR spectra were obtained from a IBM IR/45 spectrometer. A high
performance liquid chromatograph (HPLC) (Varian 5000) with a UV detector [(Vari-Chrom), 215 nm wavelength with a 16 nm slit width] was used to analyze the reaction mixtures. A 25 X 0.46 cm silica column (Rainin Microsorb) was used with a 99:1 hexane/isopropanol (oxygen free) solvent system at a flow rate of 1 mL/min. The NMR analysis using deuterated chloroform as the solvent were performed on a 200 MHz NMR. A Hewlett Packard 5890 GC-MS equipped with a 100 m X 0.25 mm SP-2560 (cyano-polysiloxane) fused silica column was used to identify the cis-trans isomers of methyl linoleate. The column was maintained isothermally at 230°C for 15 minutes. The mass range scanned was 10-500 m/z. A gas chromatograph (Varian 3740 series) equipped with an flame ionization detector and the 100m X 0.25 mm SP-2560 fused silica column maintained isothermally at 210°C was used to determine the amount of addition-elimination products formed in the reactions. The GC injection port was 250°C. Negative methane chemical ionization was performed on a Finnegan TSQ 4500 mass spectrometer using a direct exposure probe ramped to 1 amp at a rate of 10 mamp/s. Methane gas at a pressure of 0.5 Torr was used for chemical ionization. The mass range scanned in the negative mode was 44-800
m/z. The source temperature was 150°C and the manifold temperature was 100°C. Negative methane chemical ionization was also performed on a Finnegan TSQ 70 triple quadrupole mass spectrometer using a direct insertion probe. The pressure of the methane gas was 1640 mTorr.

METHODS:

The Reaction of Neat Methyl Linoleate with Nitrogen Dioxide in a Carrier Gas (97,100): The bubbler apparatus (Figure 3) was assembled in the glove bag under an atmosphere of nitrogen. The nitrogen dioxide bulb was equilibrated for one hour. The helium and air carrier gas flow rates used for the reactions were 60 mL/min and 30 mL/min. The temperature of the constant temperature bath was 37°C. A one hour blank was conducted to determine the nitrogen dioxide in carrier gas concentration. In the oxygen free reactions, ultra pure helium was used as the carrier gas and in the reactions performed in the presence of oxygen, compressed air was used as the carrier gas. The volume of thymol trap solution used in the reactions, either 100 mL or 1L, depended upon the concentration of nitrogen dioxide used in the reactions. The efficiency of the thymol trap
solutions were 70 ± 4.0% for the 100 mL trap and 83 ± 2.1% (50) for the 1L thymol trap solution. A Saltzman analysis (48) was performed on the trap solution before and after bubbling nitrogen dioxide in the trap. The volume of the thymol trap solution analyzed (0.3 mL-3 mL) depended on the amount of nitrogen dioxide that was used in the reaction. After the one hour nitrogen dioxide blank was completed, neat methyl linoleate (1.45 ± 0.07 mmoles) that had been warmed to 37°C was added to the bubbler apparatus. Nitrogen dioxide in the carrier gas was bubbled through the neat solution for one hour. The methyl linoleate reaction mixture was removed from the apparatus and analyzed by HPLC and GC analysis. The starting material was also analyzed. Carvone was used as the internal standard for HPLC analysis and methyl linelaidate was used as the internal standard for GC analysis. The response factor used to determine the hydroperoxide concentration was also used to quantify the allylic nitrite isomers by HPLC analysis. The response factor for the hydroperoxides was used because the allylic nitrite compounds decompose when the solvent is completely removed preventing an accurate allylic nitrite response factor from being determined. Both the allylic nitrite isomers and the
hydroperoxide isomers have maximum UV absorptions at approximately the same wavelength and elute at similar retention times by HPLC analysis (Chapter 3). A Saltzman analysis (48) was performed on the thymol trap solution used for the reaction. The difference in the amount of nitrogen dioxide in the thymol trap solution used as a blank and the amount of nitrogen dioxide in the thymol trap solution used for the reaction was taken as the amount of nitrogen dioxide incorporated in the methyl linoleate. For some of the lower concentrations of nitrogen dioxide, only the amount of nitrogen dioxide delivered to the methyl linoleate was used.

50:50 Methyl Palmitate/ Methyl Linoleate and Nitrogen Dioxide Reaction (98,101): A 50:50 molar solution of methyl palmitate in methyl linoleate was purged of oxygen, purified and reacted with nitrogen dioxide according to the procedure given above. Methyl palmitate/methyl linoleate (0.85 g or 1.5 mmoles of methyl linoleate) was reacted with various concentrations of nitrogen dioxide.

Aqueous Reaction of Nitrogen Dioxide and 50:50 Molar Methyl Palmitate/Methyl Linoleate (99,102): A
methyl palmitate/methyl linoleate 50:50 molar solution was prepared and purified. A pH = 7.4, 0.5 M sodium phosphate buffer (40 mL) containing DTPA at a concentration of $5 \times 10^{-4}$ g/mL was purged of oxygen with argon for one hour for the oxygen free reactions. The bubbler apparatus was assembled in the glove bag under an atmosphere of nitrogen. The helium and air carrier gas flow rates used were 60 mL/min and 30 mL/min. The nitrogen dioxide bulb was equilibrated for 1 hr and a 1 hr blank was performed using either a 100 mL or 1 L thymol trap solution. A Saltzman analysis (48) was performed to determine the nitrogen dioxide concentration in the carrier gas. The aqueous pH = 7.4, 0.5 M sodium phosphate buffer (10 mL) was added to the bubbler. Nitrogen dioxide was bubbled through the buffer for one hour. The buffer was removed and the bubbler apparatus was rinsed three times with fresh buffer solution. The buffer blank was analyzed using a Saltzman analysis (48) and the pH was checked. A Saltzman analysis (48) was also performed on the thymol trap used for the aqueous buffer blank. The difference in the amount of nitrogen dioxide in the blank thymol trap and the aqueous buffer thymol trap was taken as the amount of nitrogen dioxide incorporated in the buffer. The
amount of nitrogen dioxide in the buffer obtained by
the difference in trap solutions was the same as the
amount of nitrogen dioxide determined by a direct
Saltzman analysis (48) of the buffer solution.

After the bubbler apparatus was rinsed, 10 mL of
the buffer and 0.85 ± 0.01 g of the 50:50 molar methyl
palmitate/methyl linoleate solution (1.5 mmoles of
methyl linoleate) was added to the bubbler. The
organic methyl palmitate/methyl linoleate solution
formed a thin layer on the surface of the buffer. The
methyl palmitate/methyl linoleate solution was one-
tenth the volume of the buffer solution. Nitrogen
dioxide in either a helium or air carrier gas was
bubbled through the mixture at a flow rate of either
60 mL/min or 30 mL/min for one hour.

The organic layer was extracted from the aqueous
layer in a separatory funnel using dichloromethane
(oxygen free). The extraction was performed in the
glove bag under an atmosphere of nitrogen. The
organic layer was dried with MgSO₄, filtered and
concentrated by blowing a stream of nitrogen over the
solution. The neat reaction mixture was analyzed by
HPLC and GC analysis. Carvone was used as the
internal standard for HPLC analysis and methyl
linoleaidate was used as the internal standard for GC
analysis. The starting material was also analyzed.

A Saltzman analysis (48) was performed on the trap solution used for the reaction. The difference in the amount of nitrogen dioxide in the trap solution used for the aqueous buffer blank and the trap solution used for the reaction was taken as the amount of nitrogen dioxide incorporated in the methyl palmitate/methyl linoleate solution.

Identification and Synthesis of Products: Methyl cis-9,trans-12-octadecadienoate and methyl trans-9,cis-12-octadecadienoate isomers were identified by GC-MS (103). The retention time and EI mass spectrum of the trans,trans, isomer of methyl linoleate or methyl linelaidate and the retention time and EI mass spectrum of the cis,cis methyl linoleate were obtained and compared to the EI mass spectra of the cis,trans isomers of methyl linoleate that eluted between methyl linelaidate and methyl linoleate (Figure 4). The EI mass spectra of the isomers were identical to the EI mass spectra of methyl linoleate and methyl linelaidate. The identification of the cis,trans geometrical isomers that eluted from the column was obtained from Kobayashi (94).
Figure 4: Gas chromatogram and EI spectra of methyl linoleate isomers. (a) Methyl linelaidate. (b) The cis,trans isomers of methyl linoleate. A = methyl cis-9,trans-12-linoleate (94), B = methyl trans-9,cis-12-linoleate (94). (c) Methyl Linoleate.
Methyl 9-nitro-9,12-octadecadienoate, and methyl 13-nitro-9,12-octadecadienoate (vinyl nitro compounds) coelute by HPLC and GC analysis. These isomers were isolated by HPLC analysis from a methyl linoleate and nitrogen dioxide reaction mixture and identified collectively by NMR, IR and negative methane chemical ionization (104). The proton NMR of the mixture of isomers was compared to the proton NMR of the starting material since the structure of the olefinic region is similar. (Table 1)

Table I
Proton NMR Data of the Vinyl Nitro Compound

<table>
<thead>
<tr>
<th>Vinyl Nitro</th>
<th>Methyl Linoleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemical shifts/mult./#H</td>
<td>chem. shifts/mult./#H</td>
</tr>
<tr>
<td>A= 5.4 ppm, br, m, 3H</td>
<td>A= 5.4 ppm, br, m, 4H</td>
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<tr>
<td>B= 3.66 ppm, s, 3H</td>
<td>B= 3.66 ppm, s, 3H</td>
</tr>
<tr>
<td>C= 2.7 ppm, dd, 2H</td>
<td>C= 2.7 ppm, dd, 2H</td>
</tr>
<tr>
<td>D= 2.3 ppm, t, 2H</td>
<td>D= 2.3 ppm, t, 2H</td>
</tr>
</tbody>
</table>

The area corresponding to the number of protons in the
olefinic region was the major difference in the spectra. IR: nitro [1552 cm\(^{-1}\)], [1362 cm\(^{-1}\)], \(cis\) double bond [723 cm\(^{-1}\)], and a vinylic double bond [989 cm\(^{-1}\)] (51). Negative methane chemical ionization (NCI) was used to determine the molecular weight 339 m/z and confirm the presence of a nitro functional group by the appearance of a 46 m/z ion (52). NCI: \(M^- = 339\) m/z (9%), \([M-H]^- = 338\) m/z (30%), \([C8H13O2N]^-= 155\) m/z (<1%), \([C7H13]^-= 97\) m/z (<1%), \(NO2^- = 46\) m/z (100%). The positions of the nitro functional group were determined by the appearance of 97 m/z \([C7H13]^-\) (<1%) for the 9-nitro and 155 m/z \([C8H13O2N]^-\) for the 13-nitro.

Methyl 9-nitrato-9,12-octadecadienoate, methyl 10-nitrato-9,12-octadecadienoate, methyl 12-nitrato-9,12-octadecadienoate, methyl 13-nitrato-9,12-octadecadienoate (vinyl nitrates) also coelute by HPLC and GC analysis. These isomers were isolated by HPLC analysis and identified collectively by NMR, IR, and NCI analysis (104). The proton NMR of the vinyl nitrate was compared to the proton NMR of the methyl linoleate starting material since the structure of the olefinic region is similar. The major difference in the NMR spectra was the area corresponding to the number of protons in the olefinic region (Table II).
The IR spectrum of the isomers demonstrated the presence of a nitrate functional group \([1634 \text{ cm}^{-1}]\) assymetrical strech of NO2, \([1257 \text{ cm}^{-1}]\) symmetrical vibration, \([856 \text{ cm}^{-1}]\) strecthing of the pi bonds of the N-O linkage and \([682 \text{ cm}^{-1}]\) in NO2 bond vibration (51). The NCI was used to determine the molecular weight of 355 m/z and confirm the presence of a nitrate functional group by the appearance of a 62 m/z ion (52). The positions of the nitrate functional group was determined by 97 m/z (1%) = \([\text{C7H13}]^-\) for the 9-nitrate, 196 m/z (1%) = \([\text{C11H18O2N}]^-\) for the 10-nitrate, 282 m/z (1%) = \([\text{C15H24O4N}]^-\) for the 12-nitrate, and 155 m/z (1%) = \([\text{C8H13O2N}]^-\) for the 13-nitrate.

NCI: \(M^+ = 355 \text{ m/z (15\%)}\), \([M-H]^-= 354 \text{ m/z (9\%)}, [M-0]^-= 339 \text{ m/z (12\%)}, [M-OH]^-= 338 \text{ m/z (8\%)}, [M-H2O]^-= 337 \text{ m/z (28\%)}, \text{NO3}^- = 62 \text{ m/z (37\%)}, \text{and NO2}^- = 46 \text{ m/z (100\%)}.\)
Table II
Proton NMR Data of the Vinyl Nitrate Compound

<table>
<thead>
<tr>
<th>Vinyl Nitrate</th>
<th>Methyl linoleate</th>
</tr>
</thead>
<tbody>
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<td>chem. shift/ mult./ #H</td>
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<td>A = 5.40 ppm, br, m, 4H</td>
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<tr>
<td>B = 3.66 ppm, s, 3H</td>
<td>B = 3.66 ppm, s, 3H</td>
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<tr>
<td>C = 2.7 ppm, dd, 2H</td>
<td>C = 2.7 ppm, dd, 2H</td>
</tr>
<tr>
<td>D = 2.3 ppm, t, 2H</td>
<td>D = 2.3 ppm, t, 2H</td>
</tr>
</tbody>
</table>

The coeluting positional isomers of methyl 12-nitro-13-hydroperoxy-\textit{cis}-9-octadecenoate and methyl 9-hydroperoxy-10-nitro-\textit{cis}-12-octadecenoate were synthesized by dissolving $1.0 \times 10^{-2}$ moles of methyl linoleate in 300 mL of dry carbon tetrachloride and adding $5.6 \times 10^{-5}$ moles of N-bromosuccinimide (NBS) (53,105). The mixture was refluxed for twenty minutes and filtered. The carbon tetrachloride was evaporated using a rotary-evaporator. The tan precipitate was dissolved in 100 mL of anhydrous ether and combined...
with $5.2 \times 10^{-3}$ moles of silver nitrite (54). The mixture was protected from light and stirred for 48 hrs. The mixture was filtered and concentrated by removing the solvent with a rotary evaporator. The nitro-hydroperoxide isomers that elute after the hydroperoxides of methyl linoleate by adsorption HPLC were isolated and analyzed by NMR and NCI analysis. NMR: 6.25 ppm (br m, 1 vinyl H), 5.75 ppm (br m, 1 vinyl H), 4.9 ppm (br m, 1 alpha nitro H), 4.35 ppm (br m, 1 alpha hydroperoxide H), 3.6 ppm (s, CH$_3$O, 3H), 2.3 ppm (t, alpha carbonyl 2H), 1.8 ppm (br m, 1 allylic H), 2.05 ppm (br m, 1 allylic H). NCI: $M^- = 373 \ m/z \ (30\%), \ [M-H]^-= 372 \ m/z \ (24\%), \ [M-H2]^- = 371 \ m/z \ (50\%), \ [M-OH]^- = 355 \ m/z \ (70\%), \ [M-OOH]^- = 339 \ m/z \ (32\%), \ [C13H21O4N]^- = 255 \ m/z \ (14\%), \ [C10H17O3]^- = 185 \ m/z \ (14\%), \ NO$_2^-$ = 46 \ m/z \ (100\%). The 255 m/z (14%) = [C13H21O4N]$^-$ is indicative of the methyl 12-nitro-13-hydroperoxy-cis-9-octadecenoate isomer. The 185 m/z (14%) = [C10H17O3]$^-$ is indicative of the methyl 9-hydroperoxy, 10-nitro-cis-12-octadecenoate isomer. The nitro-hydroperoxides formed from the methyl linoleate and nitrogen dioxide reaction mixture were identified by spiking the reaction mixture with the synthesized nitro-hydroperoxide isomer mixture and by
comparing the NCI spectra of the compounds.

The methyl 13-hydroperoxy-cis-9,trans-11-octadecadienoate (13 c,t-RH), methyl 13-hydroperoxy-trans-9,trans-11-octadecadienoate (13t,t-RH), methyl 9-hydroperoxy-trans-10,cis-12-octadecadienoate (9c,t-RH), and methyl 9-hydroperoxy,trans-10,trans-12-octadecadienoate (9t,t-RH) isomers were identified by comparing the retention times and spectra to known compounds (106). The NMR, UV, GC-MS and NCI spectra are presented in chapter 3.

The methyl 13-nitrito-cis-9,trans-11-octadecadienoate (13 c,t-AN), methyl 13-nitrito-trans-9,trans-11-octadecadienoate (13 t,t-AN), methyl 9-nitrito-trans-10,cis-12-octadecadienoate (9 c,t-AN), methyl 9-nitrito-trans-10,trans-12-octadecadienoate (9 t,t-AN), methyl 13-nitro-cis-9,trans-octadecadienoate (13 c,t-AN), methyl 13-nitro-trans-9,trans-11-octadecadienoate (13 t,t-AN), methyl 9-nitro,trans-10,cis-12-octadecadienoate (9 c,t-AN), and methyl 9-nitro,trans-10,trans-12-octadecadienoate (9 t,t-AN) isomers or [allylic nitrite (nitro) isomers] were identified by comparing the NMR, IR, UV, GC-MS and NCI spectral data to corresponding hydroperoxide isomers
with similar structures (Chapter 3) (107). The allylic nitrite and nitro constitutional isomers coelute by adsorption phase HPLC.

Methyl 9-nitrato-trans-10,trans-12-octadecadienoate which elutes after the hydroperoxides of methyl linoleate was isolated and analyzed by NCI analysis (108). The NCI spectrum of the 9-nitrate was compared to the NCI spectra of the methyl 9-nitrito-trans-10,trans-12-octadecadienoate and methyl 9-nitro-trans-10,trans-12-octadecadienoate mixture. The NCI spectra of the mixture also has a trace of the 9-nitrate present from the oxidation of the 9-nitrite isomer. The position of the functional group was obtained from the detection of a 151 m/z ion in the NCI spectrum which is indicative of the fragmentation of the compound in the source to form 2,4-decadienal (Chapter 3). NCI: \[ M^+ = 355 \text{ m/z (17%)}, \quad [M-\text{NO}_2]^+ = 309 \text{ m/z (25%)}, \quad [M-\text{H}_2\text{NO}_2]^+ = 307 \text{ m/z (39%)}, \quad [M-\text{CH}_3\text{NO}_3]^+ = 277 \text{ m/z (52%)}, \quad [M-\text{C}_9\text{H}_18\text{NO}_4]^+ = 151 \text{ m/z (28%)}, \quad \text{NO}_3^- = 62 \text{ m/z (100%)}, \quad \text{NO}_2^- = 46 \text{ m/z (65\%)} .\]

**Isomerization of the 13-cis,trans-Allylic Nitrite (Nitro) Isomers in the Presence of Nitrogen Dioxide (2.7 ppm) (109):** The 13-cis,trans-allylic
nitrite(nitro) isomers \([8.3 \times 10^{-7} \text{ moles}]\) was isolated from a solution of allylic nitrite(nitro) isomers on a silica column. The allylic nitrite(nitro) isomer solution was obtained by isolating the allylic nitrite(nitro) isomers from a methyl linoleate and nitrogen dioxide reaction mixture by thin layer chromatography. A 20 X 20 cm plate with a 0.25 mm silica gel layer was used with a solvent system of 94:6 hexane/isopropanol (oxygen free). The entire separation was done in the glove bag under an atmosphere of nitrogen. The 13-cis,trans-allylic nitrite(nitro) isomer was dissolved in 0.5 mL of oxygen free dodecane (Aldrich, 99+%). The bubbler apparatus was assembled and the nitrogen dioxide bulb equilibrated for one hour. A nitrogen dioxide in helium blank was run for one hour using a 100 mL thymol trap to determine the concentration of nitrogen dioxide in the carrier gas. The concentration of nitrogen dioxide in the trap was determined by Saltzman analysis (48). The flow rate of the carrier gas was 60 mL/min and the temperature of the constant temperature bath was 25°C. The 13-cis,trans-allylic nitrite(nitro) solution was placed in the bubbler and nitrogen dioxide was bubbled through the solution for one hour. The 13-cis,trans-allylic nitrite(nitro)
solution before and after the reaction was analyzed by HPLC analysis.

**Isomerization of the 13-cis,trans-Hydroperoxide and the 13-trans,trans-Hydroperoxide in the Presence of Nitrogen Dioxide (4.1 ppm) in Helium (110):** The 13-cis,trans-hydroperoxide isomer \( [5.8 \times 10^{-6} \text{ moles}] \) and the 13-trans,trans-hydroperoxide \( [2.5 \times 10^{-6} \text{ moles}] \) was isolated from a solution of methyl linoleate hydroperoxides on a silica column. The hydroperoxide solution was obtained by isolating the hydroperoxides from a one week air oxidized methyl linoleate solution using thin layer chromatography. The conditions are given above. The 13-cis,trans and 13-trans,trans hydroperoxide isomers were dissolved in 1.1 mL and 0.94 mL of oxygen free dodecane forming 5.3 mM and a 2.6 mM solutions. The reaction was conducted according to the procedures above. The flow rate of the helium carrier gas was 60 mL/min and the temperature of the constant temperature bath was 25°C. The concentration of nitrogen dioxide in helium was 4.1 ppm and < 3ppm. The starting material and reaction mixture was analyzed using HPLC analysis.

**Reaction of Nitrite/Nitrate Anions in a Buffer**
with Methyl linoleate at 37°C in the Absence of Oxygen (111): A buffer solution containing 1.1 \times 10^{-3} \text{M} nitrite and nitrate anions was made and purged of oxygen. The concentration of nitrite/nitrate anions in the buffer is the same amount of nitrite/nitrate anions formed when 228 ppm of nitrogen dioxide in air was bubbled through a pH 7.4, 0.5M phosphate buffer to react with a 50:50 methyl palmitate/methyl linoleate solution (Table VIII). The bubbler apparatus was assembled in the glove bag under an atmosphere of nitrogen. A piece of glass tubing connected to the carrier gas tubing replaced the nitrogen dioxide bulb on the bubbler apparatus. The helium carrier gas flow rate was 60 \text{mL/min}. The temperature of the constant temperature bath was 37°C. The neat methyl linoleate was purified according to the procedures in chapter 1. The nitrite/nitrate solution (10 \text{mL}) was added to the bubbler and 1.5 mmoles of neat methyl linoleate was placed on the surface of the buffer. The mixture was bubbled with helium for one hour. The neat methyl linoleate was extracted from the aqueous layer with dichloromethane according to the procedures given above. The starting material and reaction mixture were analyzed using HPLC analysis. Methyl linoleate did not appear to react with the nitrite/nitrate
Rate Constant Determination (112): The rate constant for H-atom abstraction and addition was determined by reacting 1.5 mmoles of methyl linoleate, purified according to the procedure in chapter 1, with $8.2 \times 10^{-7} - 1.6 \times 10^{-6}$ moles of nitrogen dioxide for one hour in an amber vial equipped with a teflon stir bar and screw cap. The vial (2/3) was immersed in a constant temperature bath. The rate constants at 15°C, 26°C and 45°C were determined. The entire reaction was performed in a glove bag under an atmosphere of nitrogen. The reaction was started by adding nitrogen dioxide to the vial containing methyl linoleate that had been equilibrated in the constant temperature bath for 30 minutes. Aliquots [20 $\times 10^{-3}$ mL] were taken and diluted with 0.05 mL of carvone solution [d= $2.16 \times 10^{-4}$ g/mL] every 15 minutes and analyzed by HPLC analysis. A plot of the H-abstraction products or addition products formed vs reaction time in seconds gave a slope that was equal to the rate of the reaction. The rate constants were calculated from the following rate equation that was determined for low concentrations of nitrogen dioxide (55).
rate = k [NO₂] [alkene]
The rate constant for H-abstraction at 26°C is an average of three experiments. The activation energy and pre-exponential factor was obtained by plotting ln k vs 1/T (55). The slope was equal to Ea/R and the y-intercept was equal to ln A (55).

RESULTS

The mole percentages of products detected from the reaction of nitrogen dioxide and methyl linoleate, 50:50 methyl palmitate/methyl linoleate and buffered aqueous 50:50 methyl palmitate/methyl linoleate in helium and air reactions are presented in tables III-VIII. The mole percentages of products presented are obtained from an average of five HPLC analysis and four GC analysis of the reaction mixture. The concentration of nitrogen dioxide in parts per million in the carrier gas and the identity of the carrier gas (He or air) are listed at the top of the tables. The mole percent conversion of methyl linoleate to products is displayed on the first row of the tables. The products formed from the addition mechanism are separated from the products formed from the H-abstraction mechanism. The total percent of addition
products and H-abstraction products detected are listed after each section of products. The total moles of products detected and nitrogen dioxide reacted is listed at the bottom of the tables. The kinetic chain lengths of the reactions are presented on the last row. The kinetic chain length is defined as the moles of products formed divided by the moles of nitrogen dioxide reacted. The products detected account for $96 \pm 12$ mole % of the methyl linoleate that was consumed in the reactions.

Table III displays the mole percentages of products formed when neat methyl linoleate is reacted with various concentrations of nitrogen dioxide in ultra pure helium. Methyl linoleate, unlike methyl oleate, reacts with low concentrations of nitrogen dioxide predominately by a H-atom abstraction mechanism. The H-atom abstraction products, allylic nitrite(nitro) isomers, formed at $37^\circ$C have the trans,trans isomers predominating over the cis,trans isomers (Figure 7).

Since one of the responses detected in the lungs of animals exposed to low concentrations of nitrogen dioxide is an increase in the amount of saturated fatty acid constituents of phospholipids that comprise the cell membrane (44), methyl linoleate was diluted
with methyl palmitate in a 50:50 molar ratio. Methyl palmitate is a sixteen carbon saturated fatty acid (Figure 1) that is a constituent of phosphatidyl choline. The dilution of methyl linoleate with methyl palmitate also provided a better model for the reaction that occurs when low concentrations of nitrogen dioxide reacts with unsaturated fatty acid constituents of phospholipids in the cell membrane.

Table IV displays the mole percentages of products formed when low concentrations of nitrogen dioxide reacts with a 50:50 molar methyl palmitate/methyl linoleate solution in the absence of oxygen. When methyl linoleate is diluted with methyl palmitate, H-atom abstraction products still predominate at the lower concentrations of nitrogen dioxide. One important difference between the neat methyl linoleate system (Table III) and the 50:50 methyl palmitate/methyl linoleate system (Table IV) is the mole percent conversion of methyl linoleate to products. The amount of conversion to products decreases when methyl linoleate is diluted with methyl palmitate. This decrease in the conversion of methyl linoleate to products may be one reason an increase in the amount of saturated fatty acid constituents of lipids is detected in the lungs of animals exposed to
low levels of nitrogen dioxide. The allylic nitrite(nitro) H-abstraction products formed in the absence of oxygen also show a larger difference in the amount of the trans,trans isomers predominating over the cis,trans isomers (Figure 8).

Apparently, low concentrations of nitrogen dioxide can react with methyl linoleate, either neat or diluted with methyl palmitate, by a H-atom abstraction mechanism. However, in order for nitrogen dioxide to abstract a H-atom from polyunsaturated fatty acid constituents of the cell membrane in the lung, nitrogen dioxide must survive passing through an aqueous environment without disproportionating into nitrate and nitrite ions (56,57). In order to determine whether low concentrations of nitrogen dioxide can survive an aqueous environment and abstract a hydrogen atom from methyl linoleate, nitrogen dioxide was bubbled through 10 mL of a pH= 7.4, 0.5 M sodium phosphate buffer before coming in contact with a 50:50 molar methyl palmitate/methyl linoleate solution that was layered on the surface of the buffer.

Table V displays the mole percentages of products formed when nitrogen dioxide is bubbled through 10 mL of buffer before coming in contact with a 50:50 molar
methyl palmitate/methyl linoleate solution. The improved aqueous model system demonstrated that nitrogen dioxide can survive passing through an aqueous environment without disproportionating in order to react with methyl linoleate. The low concentrations of nitrogen dioxide appear to react exclusively by a H-atom abstraction mechanism. The cis,trans methyl linoleate addition-elimination isomers are not formed at the low concentrations of nitrogen dioxide. The allylic nitrite isomers formed are hydrolyzed to allylic alcohols (58). The H-abstraction products shown are the allylic nitro isomers and allylic alcohols that coelute by adsorption HPLC. The data in table V demonstrates that low concentrations of nitrogen dioxide will probably initiate autoxidation of polyunsaturated fatty acid components of cell membranes in the lung exclusively by a H-atom abstraction mechanism.

Since the amount of nitrogen dioxide disproportionating in the buffer appears to increase with the nitrogen dioxide concentration (Figure 5), the reaction of nitrate and nitrite ions with methyl linoleate was determined. The nitrate and nitrite ions in a pH = 7.4, 0.5M sodium phosphate buffer, oxygen free, at a concentration equivalent to exposing
Figure 5: Mole percentage of nitrogen dioxide disproportionating into nitrite and nitrate anions.
228 ppm of nitrogen dioxide to the buffer did not appear to react with methyl linoleate. Therefore, the products detected in the oxygen free aqueous nitrogen dioxide reaction with methyl linoleate are not formed from the disproportionation products of nitrogen dioxide.

The nitrogen dioxide in air reactions with methyl linoleate were performed to identify the products formed in the presence of oxygen. When air was used as the carrier gas in the nitrogen dioxide and methyl linoleate reaction, only H-abstraction products were detected. These products are displayed in table VI. The cis,trans methyl linoleate addition-elimination products and the nitro-hydroperoxide isomers of methyl linoleate were not detected. The only nitrogen containing compound detected was the 9-trans,trans allylic nitrate compound that eluted after the 9-trans,trans-hydroperoxide isomer.

In table VI, the trans,trans hydroperoxide isomers predominate over the cis,trans hydroperoxide isomers (Figure 7). The same trend in isomer ratio is exhibited in table III for the allylic nitrite (nitro) isomers. The kinetic chain length is larger in the air reactions than in the oxygen free reactions.
Figure 6:
The kinetic chain length of nitrogen dioxide and methyl linoleate (18:2) reactions in the absence and presence of oxygen. ⊘ = neat 18:2 in air, □ = aqueous 18:2/methyl palmitate (MP) in air, ▼ = 18:2/MP in air, ● = neat 18:2 anaerobic, ■ = aqueous 18:2/MP anaerobic, ▼ = 18:2/MP anaerobic.
displayed in table III (Figure 6). This increase in the kinetic chain length is caused by nitrogen dioxide initiating a chain reaction in the air reactions instead of only initiation and termination reactions occurring in the absence of oxygen.

The products formed from low concentrations of nitrogen dioxide in air reacting with a 50:50 molar methyl palmitate/methyl linoleate solution are displayed in table VII. The only difference diluting methyl linoleate with methyl palmitate produces in the nitrogen dioxide in air reactions is decreasing the kinetic chain length (Figure 6) and increasing the amount of trans,trans hydroperoxide isomers predominating over the cis,trans hydroperoxide isomers (Figure 8). This increase in the isomer ratio is also seen in the allylic nitrite(nitro) isomers displayed in table IV.

The products formed when nitrogen dioxide in air is bubbled through 10 mL of buffer and reacted with a 50:50 molar methyl palmitate/methyl linoleate solution are displayed in table VIII. The percentage of nitrogen dioxide disproportionating to nitrite and nitrate is shown at the bottom of the table. When the reaction is conducted in an aqueous system, the only difference appears to be that the 9-trans,trans-allylic
nitrate(nitro) isomer is not detected at the lower concentrations of nitrogen dioxide. The 9-trans, trans-allylic nitrate(nitro) isomer detected is probably the allylic nitro isomer since nitrates hydrolyze to alcohols in water (58).

A graph of the allylic nitrite (nitro) and hydroperoxide isomers formed in the neat methyl linoleate and 50:50 molar methyl palmitate/methyl linoleate solution as the concentration of nitrogen dioxide is increased is displayed in figures 7 and 8. The change in the cis,trans and trans,trans isomer ratio of the allylic nitrite (nitro) compounds that occurred when the methyl linoleate was diluted is identical to the change in the isomer ratio seen for the corresponding hydroperoxides.

Since the isomers of the allylic nitrite(nitro) compounds appear to isomerize in the same manner as the hydroperoxide isomers, the same mechanism for hydroperoxide isomerization proposed by Porter et al. (32) (Scheme III) was applied to the allylic nitrite(nitro) compounds. In the mechanism for hydroperoxide isomerization, the 13-cis,trans peroxyl radical isomerizes rapidly to the 9-trans,trans isomer (32). The 9-trans,trans isomer isomerizes
Figure 7: (a) Mole percentage of allylic nitrite(nitro) isomers formed when nitrogen dioxide reacts with neat methyl linoleate in the absence of oxygen. \( \nabla = 13\text{-trans,trans} \) isomer, \( \triangledown = 9\text{-trans,trans} \) isomer, \( \bullet = 13\text{-cis,trans} \) isomer, \( \bigcirc = 9\text{-cis,trans} \) isomer. (b) Mole percentage of hydroperoxides formed from nitrogen dioxide reacting with methyl linoleate in the presence of oxygen. \( \nabla = 13\text{-trans,trans} \) isomer, \( \triangledown = 9\text{-trans,trans} \) isomer, \( \bullet = 13\text{-cis,trans} \) isomer, \( \bigcirc = 9\text{-cis,trans} \) isomer.
Figure 8: (a). Mole percentage of allylic nitrite(nitro) isomers formed when nitrogen dioxide reacts with a 50:50 methyl linolate/methyl palmitate solution in the absence of oxygen. ▼ = 13-trans,trans, ◦ = 9-trans,trans, ⊙ = 13-cis,trans, ○ = 9-cis,trans.

rapidly to the 13-trans,trans isomer (32). The 13-trans,trans isomer isomerizes slowly to the 9-cis,trans isomer (32). Finally, the 9-cis,trans isomer isomerizes slowly to the 13-cis,trans isomer (32). All the isomerization steps are reversible (32).

A hydroperoxide isomer when isolated isomerized to the other isomers within a 24 hr period. However, the allylic nitrite (nitro) isomers when isolated did not isomerize. These allylic nitrite (nitro) compounds, when isolated, decomposed within a 24 hr period.

Since the allylic nitrite (nitro) compounds do not isomerize spontaneously like the hydroperoxide isomers, nitrogen dioxide was used to determine if isomerization of the allylic nitrite (nitro) isomers would occur. The 13-cis,trans allylic nitrite (nitro) isomer was isolated and reacted with nitrogen dioxide in the absence of oxygen for one hour. The nitrogen dioxide isomerized 10% of the 13-cis,trans allylic nitrite (nitro) isomer to the 13-trans,trans allylic nitrite (nitro) isomer. The 13-trans,trans allylic nitrite (nitro) isomer formed could have originated from either a mechanism similar to the Porter mechanism (32) for hydroperoxide isomerization (Scheme
Scheme V

Isomerization of the Allylic Nitrite(Nitro) Isomers
Scheme VI
Isomerization of the Allylic Nitrite (Nitro) Isomers
by an Addition-Elimination Mechanism

\[
\begin{align*}
\text{NO}_2 & \quad \text{\textbullet} \\
\text{NO}_2 & \quad \text{NO}_2 \\
\text{NO}_2 & \quad \text{NO}_2
\end{align*}
\]
V) or an addition-elimination mechanism (Scheme VI).

In order to determine which mechanism was occurring, the 13-cis,trans hydroperoxide and the 13-trans,trans hydroperoxide were isolated and reacted with nitrogen dioxide in the absence of oxygen for one hour. The nitrogen dioxide isomerized 12% of the 13-cis,trans hydroperoxide to the 13-trans,trans isomer. However, 1.7% of the 13-trans,trans hydroperoxide was converted to the 13-cis,trans isomer instead of the 9-trans,trans isomer that would have been formed if the isomerization of the isomers followed the Porter mechanism (32). Based on these experiments, the isomerization of the allylic nitrite(nitro) isomers was determined to be caused by addition-elimination of nitrogen dioxide to the double bond (Scheme VI). Addition-elimination of nitrogen dioxide to conjugated double bonds was reported earlier by Ohta et al. (62).
Table III

Mole Percentage of Products Formed from NO₂ and Methyl Linoleate Reactions in Helium at 37°C

<table>
<thead>
<tr>
<th>ppm of NO₂:</th>
<th>6.8</th>
<th>24</th>
<th>30</th>
<th>81</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2:</td>
<td>6.04</td>
<td>7.40</td>
<td>7.40</td>
<td>10.2</td>
</tr>
</tbody>
</table>

Addition Products:

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cis,trans-18:2</td>
<td>4.00</td>
<td>---</td>
<td>6.10</td>
<td>6.20</td>
</tr>
<tr>
<td>vinyl nitro</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>44.3</td>
</tr>
<tr>
<td>Total addition:</td>
<td>4.00</td>
<td>---</td>
<td>6.10</td>
<td>50.5</td>
</tr>
</tbody>
</table>

H-abstraction Products:

<table>
<thead>
<tr>
<th></th>
<th>13-c,t-AN</th>
<th>13-t,t-AN</th>
<th>9-c,t-AN</th>
<th>9-t,t-AN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.8</td>
<td>21.8</td>
<td>21.3</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>26.0</td>
<td>28.3</td>
<td>26.6</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
<td>21.2</td>
<td>19.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>26.0</td>
<td>28.7</td>
<td>26.1</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Total H-abstraction: 96.0 100 93.9 49.5

<table>
<thead>
<tr>
<th></th>
<th>moles products 10⁻⁶:</th>
<th>3.40</th>
<th>4.95</th>
<th>3.99</th>
<th>8.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>moles NO₂ 10⁻⁶:</td>
<td>0.96</td>
<td>3.3</td>
<td>4.2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>KCL(prod./NO₂):</td>
<td>3.5</td>
<td>1.5</td>
<td>0.95</td>
<td>0.74</td>
<td></td>
</tr>
</tbody>
</table>

a. The values above are an average of five HPLC analysis and four GC analysis. The uncertainty in products range from 0.04-5.4 %. The uncertainty in the moles of products and NO₂ are 4.0 X 10⁻⁶ to 1.0 X 10⁻⁶ and 5.0 X 10⁻⁸ to 3.0 X 10⁻⁷.
Table IV

Mole Percentage of Products Formed from 50:50 Methyl Palmitate/Methyl Linoleate and NO₂ Reactions in Helium at 37°C

<table>
<thead>
<tr>
<th>ppm NO₂:</th>
<th>10</th>
<th>38</th>
<th>49</th>
<th>235</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2:</td>
<td>2.50</td>
<td>3.30</td>
<td>4.40</td>
<td>8.00</td>
</tr>
<tr>
<td>Addition Products:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis,trans-18:2</td>
<td>3.40</td>
<td>2.10</td>
<td>3.20</td>
<td>7.80</td>
</tr>
<tr>
<td>vinyl nitro</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>62.6</td>
</tr>
<tr>
<td>Total addition:</td>
<td>3.40</td>
<td>2.10</td>
<td>3.20</td>
<td>70.4</td>
</tr>
</tbody>
</table>

| H-abstraction Products: | | | | |
| 13-c,t-AN | 16.8 | 15.1 | 14.5 | 3.10 |
| 13-t,t-AN | 33.6 | 33.0 | 33.4 | 11.3 |
| 9-c,t-AN | 14.7 | 15.1 | 14.5 | 2.80 |
| 9-t,t-AN | 31.5 | 34.4 | 34.6 | 12.3 |
| Total H-abstraction: | 96.6 | 97.6 | 97.0 | 29.5 |

| moles products 10⁻⁶: | 1.30 | 1.90 | 2.40 | 5.00 |
| moles NO₂ 10⁻⁶: | 1.5 | 4.2 | 3.4 | 14 |
| KCL(prod./NO₂): | 0.90 | 0.50 | 0.70 | 0.40 |
Table V
Mole Percentage of Products Formed from Aqueous 50:50 Methyl Palmitate/Methyl Linoleate and NO₂ Reactions in Helium at 37°C

<table>
<thead>
<tr>
<th>ppm NO₂:</th>
<th>4.3</th>
<th>20</th>
<th>140</th>
<th>215</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2:</td>
<td>1.60</td>
<td>1.90</td>
<td>2.80</td>
<td>5.90</td>
</tr>
<tr>
<td>Addition Products:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis,trans-18:2</td>
<td>---</td>
<td>---</td>
<td>6.40</td>
<td>4.40</td>
</tr>
<tr>
<td>vinyl nitro</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>46.7</td>
</tr>
<tr>
<td>Total addition:</td>
<td>0.0</td>
<td>0.0</td>
<td>6.40</td>
<td>51.0</td>
</tr>
</tbody>
</table>

H-abstraction Products:

| 13-c,t-AN | 16.3 | 16.7 | 14.1 | 7.40 |
| 13-t,t-AN | 34.6 | 32.9 | 32.3 | 17.4 |
| 9-c,t-AN | 14.5 | 15.4 | 12.8 | 6.60 |
| 9-t,t-AN | 34.5 | 35.4 | 34.6 | 17.4 |
| Total H-abstraction: | 99.9 | 100 | 93.8 | 49.0 |

| moles products 10⁻⁶: | 0.80 | 1.10 | 1.20 | 4.00 |
| moles NO₂ 10⁻⁶: | 0.60 | 1.4 | 1.6 | 4.6 |
| KCL(prod/NO₂): | 1.3 | 0.79 | 0.77 | 0.87 |
| % NO₂ in Buffer: | --- | 16 | 41 | 56 |
Table VI
Mole Percentage of Products Formed from Methyl Linoleate and NO$_2$ Reactions in Air at 37°C

<table>
<thead>
<tr>
<th>ppm NO$_2$:</th>
<th>2.4</th>
<th>15</th>
<th>30</th>
<th>179</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2:</td>
<td>32.0</td>
<td>41.0</td>
<td>44.0</td>
<td>63.0</td>
</tr>
</tbody>
</table>

Addition Products:

| Total addition: | 0.0 | 0.0 | 0.0 | 0.0 |

H-abstraction Products:

| 13-c,t-RH | 22.0 | 22.0 | 22.0 | 22.0 |
| 13-t,t-RH | 28.0 | 28.0 | 28.0 | 28.0 |
| 9-c,t-RH | 21.0 | 21.0 | 21.0 | 23.0 |
| 9-t,t-RH | 28.0 | 28.0 | 28.0 | 27.0 |
| 9-t,t-AN | 0.60 | 0.40 | 0.40 | 1.00 |

Total H-abstraction: 100 100 100 100

| moles products $10^{-5}$: | 2.20 | 3.00 | 3.50 | 7.50 |
| moles NO$_2$ $10^{-6}$: | 0.30 | 1.8 | 1.5 | 17 |
| KCL(prod/NO$_2$): | 79 | 17 | 23 | 4.4 |
Table VII

Mole Percentage of Products Formed from 50:50 Methyl Palmitate/Methyl Linoleate and NO₂ Reactions in Air at 37°C

<table>
<thead>
<tr>
<th>ppm NO₂</th>
<th>4.3</th>
<th>33</th>
<th>48</th>
<th>127</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2</td>
<td>18.0</td>
<td>48.0</td>
<td>44.0</td>
<td>57.0</td>
</tr>
</tbody>
</table>

Addition Products:

- vinyl nitro: --- --- --- 0.60
- vinyl nitrate: --- --- --- 4.60

Total addition: 0.0 0.0 0.0 5.20

H-abstraction Products:

- 13-c-,t-RH: 15.0 16.0 15.0 16.0
- 13-t-,t-RH: 36.0 35.0 35.0 32.0
- 9-c-,t-RH: 14.0 15.0 14.0 15.0
- 9-t-,t-RH: 35.0 35.0 36.0 32.0
- 9-t-,t-AN: --- 0.40 0.30 0.50

Total H-abstraction: 99.6 100 99.9 94.4

mole products $10^{-5}$: 0.70 2.90 2.30 3.70
mole NO₂ $10^{-6}$: 0.6 3.7 3.0 14
KCL(prod/NO₂): 12 7.8 7.7 2.6
Table VIII

Mole Percentage of Products Formed from Aqueous 50:50 Methyl Palmitate/Methyl Linoleate and NO₂ Reactions in Air at 37°C

<table>
<thead>
<tr>
<th>ppm NO₂:</th>
<th>4.3</th>
<th>29</th>
<th>43</th>
<th>228</th>
</tr>
</thead>
<tbody>
<tr>
<td>% conversion 18:2:</td>
<td>7.9</td>
<td>34</td>
<td>34</td>
<td>47</td>
</tr>
<tr>
<td>Addition Products:</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

H-abstraction Products:

<table>
<thead>
<tr>
<th></th>
<th>ppm</th>
<th>4.3</th>
<th>29</th>
<th>43</th>
<th>228</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-c,t-RH</td>
<td>15.0</td>
<td>16.0</td>
<td>16.0</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>13-t,t-RH</td>
<td>34.0</td>
<td>34.0</td>
<td>34.0</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td>9-c,t-RH</td>
<td>14.0</td>
<td>15.0</td>
<td>15.0</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>9-t,t-RH</td>
<td>36.0</td>
<td>34.0</td>
<td>34.0</td>
<td>33.0</td>
<td></td>
</tr>
<tr>
<td>9-t,t-AN</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.70</td>
<td></td>
</tr>
</tbody>
</table>

Total H-abstraction: 100 100 99.9 99.9

<table>
<thead>
<tr>
<th>moles products 10⁻⁵:</th>
<th>0.30</th>
<th>1.70</th>
<th>1.10</th>
<th>3.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>moles NO₂ 10⁻⁶:</td>
<td>0.2</td>
<td>2.1</td>
<td>1.8</td>
<td>7.8</td>
</tr>
<tr>
<td>KCL(prod/NO₂):</td>
<td>15</td>
<td>8.2</td>
<td>6.3</td>
<td>4.1</td>
</tr>
<tr>
<td>% NO₂ in Buffer:</td>
<td>---</td>
<td>26</td>
<td>9.5</td>
<td>67</td>
</tr>
</tbody>
</table>

The uncertainty in the products range from (0.04 - 5.4). The uncertainty in the moles of products and moles of NO₂ are 4.0 X 10⁻⁸ to 1.0 X 10⁻⁶ and 5.0 X 10⁻⁸ to 3.0 X 10⁻⁷.
DISCUSSION

The product study demonstrates that low concentrations of nitrogen dioxide can abstract a hydrogen atom from methyl linoleate to initiate autoxidation. In this mechanism (Scheme VII), nitrogen dioxide abstracts a hydrogen atom from the allylic position on carbon 11 of methyl linoleate forming a resonance stabilized radical (I). Radical (I) in the absence of oxygen can terminate with nitrogen dioxide forming an allylic nitrite or allylic nitro compound (II). In the presence of nitrogen dioxide, these allylic nitrite (nitro) isomers can isomerize by an addition-elimination mechanism.

If the resonance stabilized radical (I) is formed in the presence of oxygen, a peroxyl radical (III) would be formed that can isomerize to the other isomers. Radical (III) can propagate the chain reaction by abstracting a hydrogen atom from the starting material. Nitrogen dioxide can also undergo a termination reaction with radical (III) forming a peroxyl nitrate (IV) (59). These peroxyl nitrate compounds can decompose to allylic nitrates (V) (59).

The addition mechanism (Scheme VIII) which predominates at high concentrations of nitrogen
Scheme VII
Methyl Linoleate and Nitrogen Dioxide
H-Abstraction Mechanism

\[ R' = (\text{CH}_2)_3 - \text{CH}_3 \quad R = (\text{CH}_2)_6 - \text{COCH}_3 \]

\[ \text{R'} = \cdots \quad \text{R} = \cdots \]

(\text{I})

(\text{II})

(\text{III})

(\text{IV})

(\text{V})
Scheme VIII
Methyl Linoleate and Nitrogen Dioxide
Addition Mechanism

\[ R' \overset{\text{NO}_2}{\leftrightarrow} R' \overset{\text{NO}_2}{\leftrightarrow} \text{R'NO}_2 \]

\[ R' \overset{\text{NO}_2}{\leftrightarrow} R' \overset{\text{NO}_2}{\leftrightarrow} \text{R'NO}_2 \]

\[ R' \overset{\text{NO}_2}{\leftrightarrow} R' \overset{\text{NO}_2}{\leftrightarrow} \text{R'NO}_2 \]

\[ R' \overset{\text{NO}_2}{\leftrightarrow} R' \overset{\text{NO}_2}{\leftrightarrow} \text{R'NO}_2 \]
dioxide was also detected at the low concentrations of nitrogen dioxide in the absence of oxygen. In this mechanism, nitrogen dioxide can add to the double bond to form a carbon centered radical (VI). Radical (VI) can undergo an elimination reaction which can yield the starting material or the \textit{cis,trans} methyl linoleate (VII). In the absence of oxygen, radical (VI) can terminate with nitrogen dioxide forming a dinitro or nitro-nitrite compound (VIII) that can eliminate nitrous acid to form a vinyl nitro or vinyl nitrite compound (IX). In the presence of oxygen, radical (VI) can react with oxygen forming a nitro-peroxyl radical (X). The nitro-peroxyl radical is the species that initiates autoxidation in the addition mechanism by abstracting a H-atom from the starting material to form a resonance stabilized radical (I) (19).

The mole percentages of products formed in the absence of oxygen shown in tables III-V demonstrate that as the concentration of nitrogen dioxide decreases, the H-atom abstraction mechanism increases and the addition mechanism decreases.

However, as the model for nitrogen dioxide initiated autoxidation of methyl linoleate is improved, the addition mechanism is shown not to occur at the low concentrations of nitrogen dioxide studied
Figure 9: Mole percentage of allylic nitrite(nitro) H- abstraction products formed as the concentration of nitrogen dioxide is increased. • = neat methyl linoleate, ▼ = 50:50 methyl linoleate/methyl palmitate, ■ = aqueous 50:50 methyl linoleate/methyl palmitate.
(Table V). The graph in figure 9 demonstrates that as the model is improved from a neat system to an aqueous system, H-abstraction can predominate at higher concentrations of nitrogen dioxide.

The kinetic chain length in the oxygen free reactions was less than one for the 50:50 methyl palmitate/methyl linoleate and the aqueous 50:50 methyl palmitate/methyl linoleate reactions but was greater than one for the neat methyl linoleate reactions at the lowest concentrations of nitrogen dioxide studied. The kinetic chain length being greater than one is believed to be caused by the decomposition of the allylic nitrite compounds to an alkoxyl radical and nitric oxide. Alkyl nitrites were reported by Gray to be able to cleave the O-N bond to form an alkoxyl radical and nitric oxide (73). This alkoxyl radical can abstract a hydrogen atom from methyl linoleate forming a resonance stabilized radical that can terminate with another molecule of nitrogen dioxide or even nitric oxide. If the radical terminates with a molecule of nitric oxide, a nitroso compound that can rearrange to an oxime will be formed (51). The presence of an oxime was detected in this system by the appearance of an IR absorption at 1587 cm\(^{-1}\) (Chapter 3). This coincides with the study of
the reaction of nitrogen dioxide with cyclohexene in the absence of oxygen conducted by Lightsey, in which the amount of oxime formed increased as the concentration of nitrogen dioxide decreased (19).

The decrease in the kinetic chain length in the reaction of methyl linoleate with nitrogen dioxide when methyl linoleate was diluted with methyl palmitate may demonstrate that the decomposition of the allylic nitrite compounds may be induced by the presence of other allylic nitrite or nitro compounds. The allylic nitrite (nitro) isomers readily decompose when isolated from methyl linoleate and concentrated to dryness. Therefore, when methyl linoleate was diluted with methyl palmitate, the allylic nitrite (nitro) isomers were also diluted and did not decompose under the reaction conditions.

The isomerization of the allylic nitrite(nitro) isomers appears to be induced by the presence of nitrogen dioxide. The allylic nitrite (nitro) isomers do not isomerize spontaneously or upon decomposition. Therefore, the isomerization of the allylic nitrite(nitro) isomers are probably not induced by the formation of alkoxy radicals. If alkoxy radicals were the cause of the isomerization, the opposite trend in the cis,trans and trans,trans isomer ratio would
have been seen when methyl linoleate was diluted. The \textit{trans,trans} allylic nitrite(nitro) isomers would predominate over the \textit{cis,trans} isomers more in the neat methyl linoleate system than in the diluted 50:50 methyl palmitate/methyl linoleate system because the allylic nitrite(nitro) isomers would be concentrated in the neat system and have a better chance to interact.

Nitrogen dioxide is believed to isomerize the allylic nitrite(nitro) isomers by an addition-elimination mechanism. Evidence for this mechanism was obtained from the reaction of the \textit{13-cis,trans-}allylic nitrite(nitro) isomer and the \textit{13-cis,trans-}hydroperoxide isomer with nitrogen dioxide in the absence of oxygen. Both isomers formed 10-12\% of the \textit{13-trans,trans} isomer in the presence of nitrogen dioxide. When the \textit{13-trans,trans-hydroperoxide} reacted with nitrogen dioxide in the absence of oxygen, 1.7\% of the \textit{13-cis,trans} isomer was formed. If nitrogen dioxide reacted with the \textit{13-trans,trans-hydroperoxide} by a hydrogen abstraction mechanism, the \textit{9-trans,trans} isomer would have been formed instead of the \textit{13-cis,trans} isomer according to the mechanism of hydroperoxide isomerization reported by Porter et al.
(32).

The decrease in the conversion of methyl linoleate to products when methyl linoleate was diluted with methyl palmitate was caused by a decrease in the probability of nitrogen dioxide and methyl linoleate colliding in order to undergo a reaction (65).

Nitrogen dioxide was able to pass through 10ml of a pH= 7.4, 0.5M sodium phosphate buffer because the rate of dinitrogen tetroxide disproportionating to nitrite and nitrate is slow \([k = 1 \times 10^3 \text{ s}^{-1} (64)]\).

\[
\text{N}_2\text{O}_4 + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + \text{NO}_2^- + 2\text{H}^+
\]

Therefore, the reaction of nitrogen dioxide with methyl linoleate is possible at low concentrations of nitrogen dioxide where the equilibrium promotes the formation of nitrogen dioxide rather than dinitrogen tetroxide (63).

\[
2\text{NO}_2 \rightleftharpoons \text{N}_2\text{O}_4, \quad K = 1 \times 10^4 (2,3)
\]

The nitrite and nitrate ions in a 0.5 M, pH=7.4 phosphate buffer (oxygen free) did not react with methyl linoleate. Thus, the nitrite and nitrate ions formed from the disproportionation of nitrogen dioxide do not contribute to the formation of the products detected.

The explanation suggested by Lightsey (19) for
the change in the mechanism of nitrogen dioxide initiated autoxidation of cyclohexene as the concentration of nitrogen dioxide decreased can also be applied to methyl linoleate. The thermodynamically favored addition mechanism is reversible (19). The rate constant for the addition mechanism was experimentally determined to be $0.014 \text{ M}^{-1}\text{s}^{-1}$ at 299 K with an $E_a = 17.9 \text{ kcal/mole}$ and $\ln A = -34$ for methyl linoleate. The rate constant for addition is comparable to those obtained by Pitts et al. (24) for various alkenes which range from $0.0001-0.348 \text{ M}^{-1}\text{s}^{-1}$ at 298 K. The rate constant for H-abstraction was experimentally determined to be $0.013 \pm 0.003 \text{ M}^{-1}\text{s}^{-1}$ at 299 K with an $E_a = 4.9 \text{ kcal/mole}$ and $\ln A = 3.9$ for methyl linoleate. The resulting rate constant ratio $k_{\text{add}}/k_{\text{abstr}} = 1$. Although the rate constant for addition and H-abstraction are the same for methyl linoleate, the addition mechanism is rapidly reversible [$k_{\text{rev}} = 5 \times 10^4$ to $5 \times 10^5 \text{ s}^{-1}$] (24). However, at high concentrations of nitrogen dioxide more termination reactions can occur and the carbon centered radical formed in the addition mechanism is trapped before eliminating nitrogen dioxide to re-form the starting material (19). At low concentrations of nitrogen dioxide fewer termination reactions occur and the
elimination of the nitrogen dioxide to re-form the starting material is faster than the termination reaction (19). The H-abstraction mechanism predominating over the addition mechanism at low concentrations of nitrogen dioxide can be explained by comparing the activation energies of the two mechanisms. Although the rate constants for addition and H-abstraction are the same for methyl linoleate, the activation energy for H-abstraction is smaller than that for addition. Thus at low concentrations of nitrogen dioxide, H-abstraction the kinetically favored mechanism predominates over the thermodynamically favored addition mechanism.

In the presence of oxygen, the carbon centered radical is trapped by oxygen preventing elimination from occurring (19). The addition-elimination products of methyl linoleate which are the cis,trans methyl linoleate isomers were not detected in the reactions performed in the presence of oxygen (Table VI-VIII).

However, the nitro-hydroperoxides that would be formed to prevent the elimination reaction from occurring were also not detected (Tables VI-VIII). The nitro-hydroperoxides were not detected because the initiation of autoxidation by low concentrations of
nitrogen dioxide occurs more by a H-abstraction mechanism than an addition mechanism. The H-abstraction mechanism occurring more than addition can be seen from the mole percentage of the products formed when the concentration of nitrogen dioxide was decreased in the absence of oxygen (Table III-V). In the absence of oxygen, more H-abstraction products are formed than addition-elimination products of methyl linoleate demonstrating that H-abstraction is the predominant mode of nitrogen dioxide attack on methyl linoleate. Once nitrogen dioxide initiates autoxidation, nitrogen dioxide which has a Gibbs free energy change for H-abstraction of methyl linoleate calculated to be $-10.1 \pm 1.8$ kcal/mole has to compete with peroxyl radicals that have a Gibbs free energy change for H-abstraction of methyl linoleate calculated to be $-9.2 \pm 1.8$ kcal/mole that propagate the chain reaction. The Gibbs free energy change for H-abstraction were calculated from reduction potentials of $1.04$ eV for NO$_2$ (66), $0.60 \pm 0.06$ eV for methyl linoleate (from H-pentadien-1,4-yl-3)(67), and $1.0 \pm 0.06$ eV for hydroperoxides(68) using the following equation:

$$\Delta G = -23.06 \Delta E$$  \hspace{1cm} \text{(67)}

Nitrogen dioxide can also undergo termination
reactions with peroxyl radicals to form peroxynitrates (69) that can decompose to allylic nitrates (19). The mole percentage of allylic nitrates formed is shown in tables VI-VIII.

Although low concentrations of nitrogen dioxide react with methyl oleate by an addition mechanism, low levels of nitrogen dioxide react with methyl linoleate by a H-abstraction mechanism. The difference in the mode of reaction of methyl oleate and methyl linoleate proves that H-abstraction can only occur if the compound has abstractable hydrogens.

Since nitrogen dioxide can survive passing through an aqueous system to initiate autoxidation, these results demonstrate that nitrogen dioxide can survive the aqueous conditions in the lung to react with polyunsaturated fatty acid components in the cell membrane by a H-abstraction mechanism.

If nitrogen dioxide reacts by a H-abstraction mechanism in the lung, nitrous acid will be formed directly in the cell membrane (17,19). The nitrous acid formed can nitrosate amide linkages of proteins forming potent carcinogenic nitrosamides directly in the membrane (25,26). The formation of nitrosamides in the lung upon inhalation of low concentrations of nitrogen dioxide may be another reason why nitrogen
dioxide can cause the spread of cancer.
CHAPTER 3

Characterization of Allylic Nitrite (Nitro)
Derivatives of Methyl linoleate

The allylic nitrite(nitro) isomers formed from the reaction of methyl linoleate with nitrogen dioxide in the absence of oxygen were identified by comparing the UV, NMR, GC-MS, and negative chemical ionization (NCI) spectral data to the corresponding data for the hydroperoxides of methyl linoleate with known and established structural characteristics (70-72). The hydroperoxides were used to elucidate the structure of the allylic nitrite(nitro) isomers because the structures of the two types of compounds differ only in the functional group attached to the allylic carbon. Since the structures of the two types of compounds are similar, a number of the spectral techniques used to identify the allylic nitrite isomers gave similar results for both the hydroperoxide isomers and allylic nitrite (nitro) derivatives. The similarities in the spectral data simplified the characterization of the allylic nitrite(nitro) compounds. Presented are the analytical techniques used to characterize the allylic nitrite (nitro) derivatives of methyl linoleate.
EXPERIMENTAL

Materials:

Methyl linoleate (99% by GC, Sigma) was purified by eluting it through four columns of alumina (1.0 g, neutral, Aldrich) the last of which contained 0.02 g of pentetic acid (DTPA) in the tip to remove and trace metals. The purification was done in a glovebag under nitrogen.

Nitrogen dioxide was generated from dinitrogen tetroxide according to the procedures given in chapter 1.

Isopentyl nitrite (97%, Aldrich), and nitrocyclohexane (97%, Aldrich) were used as IR standards.

The hexane, isopropanol, and dichloromethane solvents (HPLC grade, Mallinckrodt) were purged of oxygen before use. Ultra pure helium (99.9999% chromatographic grade, Air Products) was used as the carrier gas for the reaction.

Instrumentation:

The UV spectra were taken on a Hewlett Packard 8451A Diode Array Spectrophotometer in a 99:1 hexane/isopropanol solvent and the IR spectra were
obtained from a IBM IR/45 spectrometer. A high performance liquid chromatograph (Varian 5000 series) with a UV detector (Vari-Chrom) set at a 215 nm wavelength with a 16 nm slit width was used to separate the hydroperoxide and allylic nitrite and nitro isomers. A 25 X 0.46 cm silica column (Rainin Microsorb) was used with a 99:1 hexane/isopropanol solvent system at a flow rate of 1 mL/min. The NMR analysis was done on a 200 MHz NMR using deuterated chloroform as the solvent. The GC chromatograms were obtained on a GC-MS (Hewlett Packard 5890) with a 20 m X 0.178 mm DB-17 (methylphenyl-polysiloxane) column. The initial 100°C column temperature was held for 5 min and ramped to 290°C at a rate of 15 C/min and held at 290°C for 10 min. The injection port temperature was 250°C with the transfer line to the mass spectrometer maintained at 280°C. The mass range scanned was 10-500 m/z. The negative methane chemical ionization was performed on a Finnegan TSQ 4500 mass spectrometer using a direct exposure probe ramped to 1 amp at a rate of 10 mamp/sec. Methane gas at a pressure of 0.5 Torr was used for chemical ionization. The mass range scanned in the negative mode was 44-800 m/z. The source temperature was 150°C and the manifold temperature was 100°C.
Methods:

Preparation of Methyl Linoleate Hydroperoxides: The hydroperoxides of methyl linoleate were formed by a one week air oxidation of methyl linoleate and separated from the starting material by thin layer chromatography using a 94:6 hexane/isopropanol solvent system. The hydroperoxides were extracted from the silica gel with dichloromethane, concentrated, and resuspended in hexane. The individual hydroperoxide isomers were separated by HPLC and analyzed by UV, NMR, GC-MS, and negative methane chemical ionization.

The Formation of the Allylic Nitrite (Nitro) Isomers: Methyl 13-nitrito-cis-9, trans-11-octadecadienoate, methyl 13-nitrito-trans-9, trans-11-octadecadienoate, methyl 9-nitrito-trans-10, cis-12-octadecadienoate, methyl 9-nitrito-trans-10, trans-12-octadecadienoate, methyl 13-nitro-cis-9, trans-11-octadecadienoate, methyl 13-nitro-trans-9, trans-11-octadecadienoate, methyl 13-nitro-cis-9, trans-11-octadecadienoate, methyl 9-nitro-trans-10, cis-12-octadecadienoate, and methyl 9-nitro-trans-10, cis-12-octadecadienoate the allylic nitrite (nitro) isomers were formed by bubbling nitrogen dioxide in helium (40 ppm) through purified methyl linoleate (neat) for one
hour. The bubbler apparatus shown in Figure 3 was used for the reaction. The flow rate of the carrier gas was 60 mL/min and the temperature of the constant temperature bath was 25°C. The entire reaction was run in a glove bag under nitrogen. The nitrogen dioxide concentration was determined by Saltzman analysis (48) of a 1 L 0.1 N sodium hydroxide and 1 mM thymol trap solution used as a blank for the reaction. The allylic nitrite (nitro) compounds were separated from the methyl linoleate starting material in a glove bag under an atmosphere of nitrogen using thin layer chromatography with a 94:6 hexane/isopropanol solvent that had been purged of oxygen. The isomers were extracted into dichloromethane. The dichloromethane was evaporated and the allylic nitrite(nitro) compounds were resuspended into hexane. The allylic nitrite (nitro) positional and geometrical isomers were isolated by HPLC and characterized by UV, IR, NMR, GC-MS and negative methane chemical ionization (NCI).

To confirm the presence of the allylic nitro isomer, the 13-cis,trans allylic nitrite(nitro) isomer formed from a 13 ppm nitrogen dioxide and methyl linoleate reaction was shaken with deionized water, separated from the aqueous layer and analyzed by NCI
The characterization of the allylic nitrite(nitro) compounds was accomplished by comparing the spectral data of the allylic nitrite(nitro) isomers with the spectral data of the corresponding hydroperoxide isomers to aid in structure elucidation.

RESULTS

The allylic nitrite(nitro) isomers elute at similar retention times to the hydroperoxides of methyl linoleate (Figure 10). When the allylic nitrite (nitro) isomers are isolated they decompose within a 12 hr period. However, the hydroperoxides of methyl linoleate isomerize to the other hydroperoxide isomers when isolated. The isomerization of methyl linoleate hydroperoxides was also reported by Chan and Levett (70).

The UV maximum absorption wavelength of the allylic nitrite (nitro) isomers and hydroperoxide isomers in a 99:1 hexane/isopropanol solution was 236 ± 2 nm for the cis,trans isomers and 234 ± 2 nm for the trans,trans isomers. This corresponds to a 234 ± 2 nm maximum absorption wavelength for the cis,trans
Figure 10: HPLC chromatograms of the allylic nitrite(nitro) isomers (a) and hydroperoxide isomers (b).
hydroperoxides and a $232 \pm 2$ nm maximum absorption wavelength for the $\text{trans,trans}$ isomers. The maximum absorption of the two types of compounds being the same demonstrates that the allylic nitrite (nitro) isomers have conjugated double bonds like the hydroperoxides of methyl linoleate.

The structural characteristics of the allylic nitrite (nitro) derivatives of methyl linoleate were determined by comparing the proton NMR spectra of the allylic nitrite (nitro) isomers to the proton NMR of the corresponding hydroperoxides of methyl linoleate. The proton assignments for the $13-\text{cis,trans}$ hydroperoxide were reported by Gardner et al. The proton assignments for the $13-\text{trans,trans}$ hydroperoxide were obtained by using a spin-spin decoupling technique. The proton NMR of the allylic nitrite(nitro) isomers have similar chemical shifts and splitting patterns to the hydroperoxides of methyl linoleate (Table IX & X).
Table IX

NMR Data for the 13-cis,trans Isomers

<table>
<thead>
<tr>
<th></th>
<th>13-c,t-allylic nitrite(nitro)</th>
<th>13-c,t-hydroperoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>chemical shift/ mult. / J</td>
<td>chemical shift/mult./ J</td>
</tr>
<tr>
<td>A</td>
<td>6.58 ppm (dd, J=10.5,15)</td>
<td>6.58 ppm (dd, J=11.4, 15.7)</td>
</tr>
<tr>
<td>B</td>
<td>6.02 ppm (t, J=10.5,12.5)</td>
<td>6.02 ppm (t, J=10.7,11.4)</td>
</tr>
<tr>
<td>C</td>
<td>5.5 ppm (m, J=9)</td>
<td>5.5 ppm (m, J=8.5)</td>
</tr>
<tr>
<td>D</td>
<td>4.4 ppm (m, J=7.5)</td>
<td>4.4 ppm (m, J=7.8)</td>
</tr>
</tbody>
</table>
Table X

NMR Data for the 13-trans.trans Isomers

<table>
<thead>
<tr>
<th></th>
<th>13-t,t-allylic nitrite(nitro)</th>
<th>13-t,t-hydroperoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>chemical shift/mult./ J</td>
<td>chemical shift/mult./ J</td>
</tr>
<tr>
<td>A= 6.29 ppm (dd, J= 10.5,15.5)</td>
<td>A=6.28 ppm(dd, J= 11.6,16)</td>
<td></td>
</tr>
<tr>
<td>B= 6.05 ppm (t, J= 15,12.5)</td>
<td>B=6.0 ppm(t, J= 11.6,16.8)</td>
<td></td>
</tr>
<tr>
<td>C= 5.77 ppm (dd, J= 6.5,15.5)</td>
<td>C=5.8 ppm(dd, J= 7.7,16.1)</td>
<td></td>
</tr>
<tr>
<td>D= 5.47 ppm (dd, J= 9, 15)</td>
<td>D=5.45 ppm (dd, J= 9.6,16.1)</td>
<td></td>
</tr>
<tr>
<td>E= 4.34 ppm (m, J= 7.5)</td>
<td>E= 4.35 ppm (m, J= 7.7)</td>
<td></td>
</tr>
</tbody>
</table>

The 9-positional allylic nitrite(nitro) isomers have the same chemical shift and splitting pattern as the 13-positional isomers of the allylic nitrite(nitro) compounds. (Table XI & XII)
Table XI

NMR Data of the 9-Allylic Nitrite(Nitro) Isomers

<table>
<thead>
<tr>
<th></th>
<th>9-c,t-allylic nitrite(nitro)</th>
<th>9-t,t-allylic nitrite(nitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>chemical shifts/mult./ J</td>
<td>chemical shifts/mult./ J</td>
</tr>
<tr>
<td>A</td>
<td>6.58 ppm, (dd, J = 10.5,15)</td>
<td>6.29 ppm (dd, J = 10.5,15.5)</td>
</tr>
<tr>
<td>B</td>
<td>6.02 ppm, (t, J=10.5,12.5)</td>
<td>6.05 ppm (t, J = 15, 12.5)</td>
</tr>
<tr>
<td>C</td>
<td>5.5 ppm, (m, J = 9)</td>
<td>5.77 ppm (dd, J = 6.5, 15.5)</td>
</tr>
<tr>
<td>D</td>
<td>4.4 ppm, (m, J = 7.5)</td>
<td>5.47 ppm (dd, J = 9, 15)</td>
</tr>
<tr>
<td>E</td>
<td>4.34 ppm (m, J = 7.5)</td>
<td></td>
</tr>
</tbody>
</table>

The similarities of the chemical shifts and splitting patterns for both types of compounds' proton NMR spectra demonstrates that the compounds have similar structural geometries. The cis,trans and trans,trans geometries of the isomers are also clearly distinguished by proton NMR analysis.
The positions of the nitrite functional group on the allylic nitrite (nitro) isomers were determined using GC-MS analysis. The allylic nitrite (nitro) isomers thermal decomposition products were compared to the thermal decomposition products of the corresponding hydroperoxides to confirm and locate the position of the functional group.

Hydroperoxides are known to undergo thermal homolysis of the O-O bond to form an intermediate alkoxy radical that can subsequently undergo carbon-carbon bond scission to form an aldehyde (71-72) (Scheme IX & X). The aldehydes formed from carbon-carbon scission can be used to locate the position of the hydroperoxide functional group.

The allylic nitrite isomers can also undergo thermal homolysis of the O-N bond to form an alkoxy radical (73) that can undergo carbon-carbon scission (Scheme IX & X). If the positions of the nitrite functional group of the allylic nitrite isomers are the same as the positions of the hydroperoxy functional group of the hydroperoxides, the same aldehydes should be formed upon thermal homolysis. The chromatograms in Figure 11 show the thermal decomposition products of the hydroperoxides and the allylic nitrite(nitro) isomers.
Scheme IX

The Formation of 2,4-Decadienal from Thermal Homolysis of the 9-Hydroperoxide and 9- Allylic Nitrite Isomers of Methyl Linoleate

\[
\begin{align*}
\text{9-CIS,TRANS-HYDROPEROXIDE} \\
\text{9-CIS,TRANS-ALLYLIC NITRITE}
\end{align*}
\]
Scheme X

The Formation of Methyl 13-Oxo-9,11-Tridecadienoate from Thermal Homolysis of the 13-Hydroperoxide and 13-Allylic Nitrite Isomers of Methyl Linoleate

13-CIS,TRANS-HYDROPEROXIDE

13-CIS,TRANS-ALLYLIC NITRITE
One of the aldehydes known to be formed from thermal homolysis of the 9-cis,trans and 9-trans,trans hydroperoxides is 2,4-decadienal (Scheme IX) (72). The 2,4-decadienal isomers appear at a retention time (r.t.) of 9.8 and 10.2 in both of the chromatograms for the 9-hydroperoxide and 9-allylic nitrite(nitro) compounds shown in figures 11a-11d. The 2,4-decadienal isomers were identified by comparing the mass spectrum to the National Bureau of Standards mass spectrum for 2,4-decadienal. Mass Spectrum (EI): M=152 m/z, [M-C4H9]= 95 m/z and [M-C5H11]= 81 m/z. The elution order of the isomers was also compared to the elution order obtained by Chan et al. (72) for 2,4-decadienal isomers under similar conditions.

One of the aldehydes reported by Frankel et al. (74) to be formed when the 13-cis,trans and 13-trans,trans-hydroperoxides undergo homolysis is methyl 13-oxo-9,11-tridecadienoate (Scheme X). The methyl 13-oxo-9,11-tridecadienoate isomers appear at a r.t. of 16.3 and 16.5 in both of the chromatograms for the 13-hydroperoxide and 13-allylic nitrite(nitro) compounds shown in figures 11e-11h. Methyl 13-oxo-9,11-tridecadienoate was tentatively identified by its mass special fragmentation pattern. Mass spectrum
Figure 11: Gas chromatograms of methyl linoleate hydroperoxides and allylic nitrite(nitro) isomers. The arrows depict the 2,4-decadienal isomers (a-d) and the methyl 13-oxo-9,11-tridecadienoate isomers (e-h) formed from thermal homolysis.
(EI): \([\text{M-CH}_3\text{OH}] = 206 \text{ m/z}, [\text{M-C}_2\text{H}_3\text{O}_2] = 189 \text{ m/z}, \) and \([\text{M-C}_9\text{H}_17\text{O}_2] = 81 \text{ m/z} \).

The negative methane chemical ionization spectra for the hydroperoxides is displayed in Figures 12-15. The NCI spectra for the hydroperoxides show an \((\text{M-H})^-\) peak of 325 m/z (Figure 12 & 13). The molecular ion 326 m/z was not detected. The \((\text{M-H})^-\) ions of 151 m/z for 2,4-decadienal and 237 m/z for methyl 13-oxo-9,11-tridecadienoate homolysis products of the 9 and 13-hydroperoxides (Scheme IX & X) was also seen in the NCI spectra of the 9 and 13-hydroperoxides (Figures 12 & 13). The 151 m/z ion displayed in the NCI spectra of the 9-\text{cis,trans} and 9-\text{trans,trans}-hydroperoxides was not seen in the NCI spectra of the 13-\text{cis,trans} and 13-\text{trans,trans}-hydroperoxides. The 237 m/z ion detected in the NCI spectra of the 13-\text{cis,trans} and 13-\text{trans,trans}-hydroperoxides was also not seen in the NCI spectra of the 9-\text{cis,trans} and 9-\text{trans,trans} hydroperoxides. The 151 m/z and 237 m/z ion of 2,4-decadienal and methyl 13-oxo-9,11-tridecadienoate in the NCI spectra of the hydroperoxides can also be used to locate the position of the hydroperoxide functional group.

The NCI spectra of the allylic nitrite(nitro)
Figure 12: Negative methane chemical ionization spectra of the 9-hydroperoxide isomers. (a). 9-cis,trans-hydroperoxide. (b). 9-trans,trans-hydroperoxide.
Figure 13: Negative methane chemical ionization spectra of the 13-hydroperoxides. (a). 13-cis,trans-hydroperoxide. (b). 13-trans,trans-hydroperoxide.
Figure 14: Negative methane chemical ionization spectra of the 9-allylic nitrite(nitro) isomers. (a). 9-cis,trans allylic nitrite(nitro). (b). 9-trans,trans-allylic nitrite(nitro).
Figure 15: Negative methane chemical ionization spectra of the 13-allylic nitrite(nitro) isomers. (a). 13-cis,trans-allylic nitrite(nitro) isomer. (b). 13-trans,trans-allylic nitrite(nitro) isomers.
isomers are displayed in Figures 14 & 15. The molecular ion of 339 m/z the (M-H)', ion of 338 m/z and a (M+H)' ion of 340 m/z are present in the NCI spectra of the allylic nitrite(nitro) isomers. The 46 m/z ion indicative of a NO2 functional group (76-78) and a 62 m/z ion indicative of a NO3 functional group (76-78) are also present in the NCI spectra of the allylic nitrite(nitro) isomers. The 46 m/z and the 62 m/z ions were not detected in the NCI spectra of the hydroperoxides. The (M-H)' ions of 151 m/z and 237 m/z for the 2,4-decadienal and methyl 13-oxo-9,11-tridecadienoate homolysis products of the 9-allylic nitrite and the 13-allylic nitrite isomers are also displayed in the NCI spectra of the allylic nitrite(nitro) compounds (Figure 14 & 15).

The allylic nitro isomer was demonstrated to coelute by HPLC with the allylic nitrite isomer by hydrolysis of the 13-cis,trans-allylic nitrite(nitro) compound with subsequent NCI analysis. Hydrolysis of the 13-cis,trans allylic nitrite(nitro) isomer and subsequent NCI analysis displayed the 46 m/z ion and 62 m/z ion indicative of the nitro and nitrate functional group (76-78) and the (M-H)' 338 m/z and (M+H)' 340 m/z ions. If the sample consisted of only
the allylic nitrite isomer, the 46 m/z, 62 m/z, 338
m/z and 340 m/z ions would not have been detected upon
hydrolysis of the sample because alkyl nitrites are
known to hydrolyze to alcohols (75).

The IR spectral data for the allylic
nitrite(nitro) isomers is shown in table XII. The IR
stretching frequencies of 1603 and 1587 cm⁻¹ of the
allylic nitrite(nitro) isomers may be caused by the
presence of an oxime (126) formed from the
decomposition of the allylic nitrite isomers. The 323
m/z ion in the NCI spectra of the allylic
nitrite(nitro) compounds may be the M⁺ ion of the
oxime compound. The stretching frequency for the
nitro functional group is 1377 cm⁻¹. The stretching
frequencies diverging from the normal values may be
cau sed by the electron withdrawing effects of the
double bonds (29) on the compound and the sample being
a mixture of nitrite and nitro isomers. Table XII
displays the functional group IR frequencies for
isopentyl nitrite, nitrocyclohexane, and a mixture of
isopentyl nitrite and nitrocyclohexane. When the
nitrite compound and nitro compounds are together,
some of the stretching frequencies shift.
Table XII

IR Stretching Frequencies cm\(^{-1}\) of Nitro and Nitrite Compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allylic nitrite(nitro) isomers</td>
<td>1603, 1587, 1377</td>
</tr>
<tr>
<td>Isopentyl nitrite</td>
<td>1639.7, 1552.9</td>
</tr>
<tr>
<td>Nitrocylohexane</td>
<td>1545, 1379</td>
</tr>
<tr>
<td>Isopentyl nitrite &amp; Nitrocylohexane</td>
<td>1653, 1635, 1545, 1377</td>
</tr>
</tbody>
</table>

DISCUSSION

The UV data of the hydroperoxides and allylic nitrite compounds demonstrate that the allylic nitrite(nitro) compounds have conjugated double bonds. The maximum absorbances of the isomers are practically the same within experimental error.

The proton NMR data of the hydroperoxides and allylic nitrite(nitro) compounds show that the two types of compounds are structurally similar. The chemical shifts of the two types of compounds and splitting patterns are practically the same. However, some of the coupling constants of the hydroperoxides...
and allylic nitrite(nitro) compounds are different. The cis,trans and trans,trans geometries of the allylic nitrite isomers are also clearly distinguished by using proton NMR analysis. The comparison of the proton NMR of the allylic nitrite(nitro) isomers to the proton NMR of the hydroperoxide isomers enabled the structures of the allylic nitrite(nitro) isomers to be elucidated.

The positions of the functional groups for the allylic nitrite(nitro) compounds were obtained by the detection of the 2,4-decadienal and methyl 13-oxo-9,11-tridecadienoate homolysis products. Hydroperoxides were reported by Chan et al. to undergo thermal homolysis of the O-O bond of the hydroperoxide to form an alkoxy radical (72) with subsequent carbon-carbon bond scission to form aldehydes (72). The aldehydes known to be formed from the carbon-carbon bond scission of the 9 and 13-hydroperoxides were used to confirm the position of the hydroperoxy functional group.

Alkyl nitrites have also been reported to undergo thermal homolysis of the O-N bond to form alkoxy radicals and nitric oxide (73). Since the allylic nitrites can also form the same intermediate alkoxy radical that the hydroperoxides form, the same
aldehydes formed by the hydroperoxides were demonstrated using GC-MS and NCI analysis to be formed by the allylic nitrite isomers. The presence of 2,4-decadienal in both the 9-allylic nitrite and 9-hydroperoxide isomers gc chromatogram (Figures 11) and NCI spectra (Figures 12-15) confirmed the position of the functional group. The presence of methyl 13-oxo-9,11-tridecadienoate in both the 13-allylic nitrite and 13-hydroperoxide isomers gc chromatogram (Figures 11e-11h) and NCI spectra (Figures 12-15) was used to confirm the position of the functional group. The trace amount of 2,4-decadienal in the gc chromatogram of the 13-hydroperoxides is reported by Chan et al. (72) to be caused by isomerization of the hydroperoxide in the GC injection port.

Negative methane chemical ionization was used to confirm the molecular weight of the allylic nitrite(nitro) and hydroperoxide compounds. The M⁻ ion of 339 m/z was detected in the NCI spectra of the allylic nitrite(nitro) compounds but an M⁻ ion was not detected for the hydroperoxides. The M⁻ ion is formed by resonance capture and the presence of an M⁻ ion is a reflection of the ability of the compound to stabilize a negative charge (77).

The (M-H)⁻ ions of 338 m/z and 325 m/z detected
in the NCI spectra for the allylic nitrite(nitro) compounds and the hydroperoxides is caused by dissociative electron capture (77). The formation of (M-H)⁻ ions are characteristic for NCI mass spectrometry (77,79).

An (M+H)⁺ ion of 340 m/z was detected in the NCI spectra of the allylic nitrite(nitro) compounds but an (M+H)⁻ ion was not detected in the NCI spectra of the hydroperoxides. The (M+H)⁻ ion is formed from ion molecule reactions of M⁻ abstracting a hydrogen atom (80). The formation of (M+H)⁻ ions were reported by Dougherty et al. to be formed for all chlorinated compounds studied that formed a molecular anion (80).

The 46 m/z ion present in the NCI spectra for the allylic nitrite(nitro) compounds is indicative of a nitro functional group (76-78). The 46 m/z ion is formed from dissociative electron capture and is a characteristic ion observed for nitro-containing compounds (76-78).

The 62 m/z ion shown in the NCI spectra of the allylic nitrite(nitro) compounds is indicative of a nitrate functional group (76-78). The 62 m/z is believed to originate from homolysis of the allylic nitrite to an alkoxy radical in the source with
subsequent termination of the radical with NO2 to form an allylic nitrate. The alkoxy radical is known to be formed because the 151 m/z and 237 m/z (M-H)⁻ ions of the aldehydes formed from carbon-carbon homolysis of the allylic nitrite alkoxy radical intermediate were detected. The 62 m/z ion is believed to be formed in the source rather than originating from an allylic nitrate present in the sample because a 355 m/z ion indicative of the M⁻ ion for an allylic nitrate was not detected.

The allylic nitro compounds were demonstrated to coelute with the allylic nitrite compounds by hydrolysis of the 13-cis, trans allylic nitrite (nitro) compound. Alkyl nitrites are known to hydrolyze to the corresponding alcohol in the presence of water (75). The hydrolysis reaction with subsequent NCI analysis demonstrated that the allylic nitro compound was present in the sample. If the sample consisted of only the allylic nitrite isomer, the NCI spectra of the hydrolyzed sample would not have contained the 46 m/z, 62 m/z, 338 m/z, and 340 m/z ions because the allylic nitrite compound would have hydrolyzed to the allylic alcohol (75).

The IR analysis demonstrated that the allylic nitrite isomers can probably decompose into an oxime.
The presence of a nitro functional group was also confirmed by the IR analysis.

CONCLUSION

The allylic nitrite(nitro) derivatives of methyl linoleate formed from the reaction of a low concentration of nitrogen dioxide with methyl linoleate were characterized using the hydroperoxides of methyl linoleate as a model. The data presented demonstrate that the structures of the two types of compounds are very similar differing only in the functional group present.

The characterization of the allylic nitrite(nitro) derivatives of methyl linoleate formed from the reaction of a low concentration of nitrogen dioxide with methyl linoleate also demonstrates that nitrogen dioxide can react by a H-abstraction mechanism.
CHAPTER 4

Reaction of Nitrogen Dioxide with a 50:50 Molar Methyl Oleate & Methyl Linoleate Solution

Earlier, methyl oleate was demonstrated to react with a low concentration of nitrogen dioxide by an addition mechanism. However, methyl linoleate was shown to react with low concentrations of nitrogen dioxide predominantly by a H-abstraction mechanism. Since both unsaturated fatty acids are constituents of phosphatidylcholine, a pulmonary lipid that comprises cell membranes, a study was performed to determine whether a low concentration of nitrogen dioxide would react with a 50:50 molar methyl oleate/methyl linoleate solution by either an addition or a H-abstraction mechanism (113). The 50:50 molar ratio of methyl oleate/methyl linoleate is the same molar ratio found in phosphatidyl choline of human erythrocytes (81).

Experimental

Materials:

Methyl oleate (3.1 mmoles, 99% by GC from Sigma)
and methyl linoleate (3.1 mmoles, 99% by GC from Sigma) were combined and purified by passing the solution through four columns of alumina (1 g, neutral, Aldrich). The last column contained pentetic acid (0.02 g) in the tip.

The solvents, instrumentation and product identification are presented in chapters 1 and 2.

Methods:

The bubbler apparatus was assembled in the glovebag under an atmosphere of nitrogen. A one hour blank was performed to determine the concentration of nitrogen dioxide in the carrier gas and analyzed by Saltzman analysis. The ultra pure helium carrier gas flow rate was 60 mL/min. The temperature of the constant temperature bath was 37°C. The 50:50 molar methyl oleate/methyl linoleate solution (0.792 g or 1.40 mmoles of methyl linoleate) was added to the bubbler. Nitrogen dioxide in helium (5 ppm) was bubbled through the solution for one hour. The reaction mixture and starting material were analyzed by HPLC and GC analysis.
Results

The HPLC chromatogram of the starting material and reaction mixture are displayed in figure 16. The GC analysis did not show the formation of any addition-elimination products from either the methyl oleate or methyl linoleate. The percentage of methyl linoleate converted to products was 3.3 ± 0.1%. The amount of H-abstraction was 99.8 ± 1.7%. The kinetic chain length (moles of products/moles of NO$_2$ reacted) was 1.7 ± 0.2.

Table XIII

Mole Percentage of Products Formed from a 50:50 Molar Methyl Oleate/Methyl Linoleate Reaction with 5 ppm of Nitrogen Dioxide (in Helium)

<table>
<thead>
<tr>
<th>H-abstraction products</th>
<th>Mole percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-cis,trans-allylic nitrite(nitro)</td>
<td>17.7 ± 0.5</td>
</tr>
<tr>
<td>13-trans,trans-allylic nitrite(nitro)</td>
<td>34.0 ± 0.6</td>
</tr>
<tr>
<td>9-cis,trans-allylic nitrite(nitro)</td>
<td>16.3 ± 0.7</td>
</tr>
<tr>
<td>9-trans,trans-allylic nitrite(nitro)</td>
<td>31.8 ± 1.4</td>
</tr>
</tbody>
</table>
Figure 16: HPLC chromatogram of the starting material (a) and reaction mixture (b) of a 50:50 methyl oleate/methyl linoleate and nitrogen dioxide reaction mixture.
The mole percentages presented are an average of five HPLC analysis.

Discussion

The results demonstrate that 5 ppm of nitrogen dioxide reacts with a 50:50 molar methyl oleate/methyl linoleate solution exclusively by a H-abstraction mechanism. The addition-elimination products of either methyl oleate or methyl linoleate were not formed. The H-abstraction products detected originated from only methyl linoleate reacting with nitrogen dioxide. H-abstraction was also found to be the only mechanism occurring when 4.3 ppm of nitrogen dioxide was bubbled through an aqueous 50:50 molar methyl palmitate/methyl linoleate solution. (Chapter 2, Table V) Apparently, at concentrations of nitrogen dioxide below 5 ppm only H-abstraction occurs.

Nitrogen dioxide reacting with methyl linoleate instead of methyl oleate can be explained by the change in the Gibbs free energy for H-abstraction. The Gibbs free energy for H-abstraction can be calculated using the reduction potentials of NO2 (1.04 eV) (66), methyl linoleate 0.60 eV (for H-pentadien-1,4-yl-3) (67) and methyl oleate 0.96 eV (for H-allyl
in propene) (67) and the following formula (67):

$$\Delta G = -23.06 \Delta E$$

The change in the Gibbs free energy for nitrogen dioxide abstracting a hydrogen atom from methyl oleate is $\Delta G = -1.8 \pm 1.8$ kcal/mole whereas the change in the Gibbs free energy for methyl linoleate is $\Delta G = -10.0 \pm 1.8$ kcal/mol. Based on the thermodynamic calculations, nitrogen dioxide should spontaneously react with methyl linoleate by a H-abstraction mechanism faster than methyl oleate. Therefore, at the low concentration of nitrogen dioxide studied in which the H-abstraction mechanism is the predominant mode of attack, nitrogen dioxide reacted with methyl linoleate instead of methyl oleate.

Consequently, if low concentrations of nitrogen dioxide are exposed to phosphatidyl choline or any lipid containing oleic acid and linoleic acid constituents in the cell membrane, nitrogen dioxide will only react with linoleic acid exclusively by a H-atom abstraction mechanism to initiate autoxidation.
CHAPTER 5
Nitrogen dioxide Reaction with Methyl Linolenate

Methyl linolenate is an essential 18-carbon polyunsaturated fatty acid with three double bonds used by the body to synthesize arachidonic acid, a 20-carbon fatty acid with four double bonds (82). Methyl linolenate was also predicted by Lightsey to react with low levels of nitrogen dioxide by a H-abstraction mechanism (19). Since low concentrations of nitrogen dioxide reacts with methyl linoleate with a $k_{\text{abstr}}/H = 31$ (49) predominantly by a H-abstraction mechanism, a low concentration of nitrogen dioxide should also react with methyl linolenate $k_{\text{abstr}}/H = 59$ (49) by a H-abstraction mechanism. In order to determine if methyl linolenate will react with nitrogen dioxide by a H-abstraction mechanism, a low concentration of nitrogen dioxide in the absence of oxygen was reacted with methyl linolenate.

Experimental

Materials:

Methyl linolenate (99 % by GC, Sigma) was
purified according to the procedure given in chapter 2. The reagents used for the product analysis are also given in chapter 2.

Instrumentation:

The GC-MS, NMR, UV and Negative Methane Chemical Ionization Mass Spectrometry used and conditions are given in chapter 3. The GC and conditions used are given in chapter 2.

Methods:

Hydroperoxides of Methyl Linolenate (114):

Methyl 16-hydroperoxy-cis-9,cis-12,trans-14-octadecatrienoate (16-c,t-RH), methyl 16-hydroperoxy-cis-9,trans-12,trans-14-octadecatrienoate (16-t,t-RH),
methyl 12-hydroperoxy-cis-9,trans-13,cis-15-octadecatrienoate (12-c,t-RH), methyl 12-hydroperoxy-cis-9, trans-13,trans-15-octadecatrienoate (12-t,t-RH),
methyl 9-hydroperoxy-trans-10,cis-12,cis-15-octadecatrienoate (9-c,t-RH), and methyl 9-hydroperoxy-trans-10,trans-12,cis-15-octadecatrienoate
(9-t,t-RH) hydroperoxides of methyl linolenate were obtained from an air oxidized methyl linolenate sample. The hydroperoxides were isolated from the methyl linolenate starting material according to the procedures given in chapter 3. The hydroperoxides of methyl linolenate are not resolved by adsorption phase HPLC. The HPLC conditions are given in chapter 3. The peaks of unresolved hydroperoxides were isolated and analyzed by UV, NMR, and GC-MS. [The hydroperoxides of methyl linolenate decompose when the solvent is completely removed.]

Since the hydroperoxide isomers are not resolved, a sodium borohydride reduction (83) was performed on the solution of methyl linolenate hydroperoxides and on the individually isolated hydroperoxide peaks. The reduction of methyl linolenate hydroperoxides with sodium borohydride was used by Chan and Levett (83) to resolve the hydroperoxides by adsorption phase HPLC analysis. The assignments for the methyl linolenate alcohols reported by Chan and Levett were used to identify the methyl linolenate alcohols obtained from the sodium borohydride reduction (83). The retention times of the individually reduced hydroperoxide alcohol products were compared to the mixture of reduced hydroperoxide alcohol product peaks to
determine the identity of the hydroperoxides contained in each unresolved hydroperoxide peak of methyl linolenate.

Sodium Borohydride Reduction (115): The hydroperoxide peaks labeled I, II, III, and IV (Figure 17) were isolated from the methyl linolenate hydroperoxide mixture and reduced to alcohols using sodium borohydride (83). The hydroperoxides were formed from an air oxidation of methyl linolenate. The mixture of hydroperoxide peaks (I-IV) (1 mg) or the individual peaks in a minimum amount of ethanol (absolute) were added to a cold mixture of 0.01 g of NaBH4 in 5 mL of absolute ethanol. The solution was stirred for 15 minutes and warmed to 25°C. The solution (room temperature) was stirred for 5 minutes and chilled again with a ice-water bath. Hydrochloric acid (3N) was added dropwise to the solution until the evolution of hydrogen ceased. The alcohol(s) of methyl linolenate were extracted with dichloromethane, dried with magnesium sulfate, filtered and concentrated with a rotary-evaporator. Hexane was added to the concentrated alcohol(s) and the solution was analyzed by adsorption phase HPLC analysis.
Figure 17: HPLC chromatograms of linolenate hydroperoxides (a) and sodium borohydride reduced linolenate hydroperoxides (b). Linolenate alcohols: A = 13-cis,trans, B = 12-cis,trans, C = 12-trans,trans & 13-trans,trans, D = 16-cis,trans, E = 9-cis,trans, F = 16-trans,trans, G = 9-trans,trans (83).
Allylic Nitrite (Nitro) Isomers of Methyl Linolenate (116): Methyl 16-nitrito-cis-9,cis-12,trans-14-octadecatrienoate (16-c,t-AN), methyl 16-nitro-cis-9,cis-12, trans-14-octadecatrienoate (16-c,t-AN), methyl 16-nitrito-cis-9,trans-12,trans-14-octadecatrienoate (16-t,t-AN), methyl 16-nitro-cis-9,trans-12,trans-14-octadecatrienoate (16-t,t-AN), methyl 13-nitrito-cis-9,trans-11,cis-15-octadecatrienoate (13-c,t-AN), methyl 13-nitro-cis-9,trans-11,cis-15-octadecatrienoate (13-c,t-AN), methyl 13-nitrito-trans-9,trans-11,cis-15-octadecatrienoate (13-t,t-AN), methyl 13-nitro-trans-9,trans-11,cis-15-octadecatrienoate (13-t,t-AN), methyl 12-nitrito-cis-9,trans-13,cis-15-octadecatrienoate (12-c,t-AN), methyl 12-nitro-cis-9,trans-13,cis-15-octadecatrienoate (12-c,t-AN), methyl 12-nitrito-cis-9,trans-13,trans-15-octadecatrienoate (12-t,t-AN), methyl 12-nitro-cis-9,trans-13,trans-15-octadecatrienoate (12-t,t-AN), methyl 9-nitrito-trans-10,cis-12,cis-15-octadecatrienoate (9-c,t-AN), methyl 9-nitro-trans-10,cis-12,cis-15-octadecatrienoate (9-c,t-AN), methyl 9-nitrito-trans-10,trans-12,cis-15-octadecatrienoate (9-t,t-AN), and methyl 9-nitro-trans-10,trans-12,cis-15-
octadecatrienoate (9-\(t,t\)-AN) or the allylic nitrite (nitro) isomers of methyl linolenate were formed by reacting purified methyl linolenate with 3 ppm of nitrogen dioxide in ultra pure helium for one hour. The procedure is given in chapter 3. The allylic nitrite (nitro) isomers were separated from the starting material by thin layer chromatography using a silica gel plate and 96:4 hexane/isopropanol oxygen free solvent system. The entire procedure was performed in a glovebag under a nitrogen atmosphere. Dichloromethane was used to extract the allylic nitrite(nitro) isomers from the silica gel. The allylic nitrite(nitro) isomers in dichloromethane were concentrated with a rotary evaporator. A minimum amount of hexane was added to the concentrated allylic nitrite isomers and the isomers were separated by HPLC analysis. The allylic nitrite (nitro) isomers were analyzed by NMR, GC-MS, and negative methane chemical ionization. [The allylic nitrite (nitro) isomers of methyl linolenate decompose when the solvent is completely removed.] The allylic nitrite (nitro) isomers of methyl linolenate were identified by comparing the allylic nitrite (nitro) isomers spectral data to the spectral data of the corresponding hydroperoxides.
Addition-Elimination Product of Methyl Linolenate (117): Methyl \textit{trans}-9,\textit{cis}-12,\textit{cis}-15-octadecatrienoate, methyl \textit{cis}-9,\textit{trans}-12,\textit{cis}-15-octadecatrienoate, and methyl \textit{cis}-9,\textit{cis}-12,\textit{trans}-15-octadecatrienoate or (c,t-18:3) that are believed to coelute by gc were tentatively identified by GC-MS analysis. The GC-MS conditions are in chapter 2. Since methyl oleate and methyl linoleate both have \textit{trans} and \textit{cis} addition-elimination products eluting before but adjacent to the starting material, the peak eluting before but adjacent to the methyl linolenate peak in the reaction mixture that has an EI spectrum identical to that of methyl linoleate was tentatively identified as the \textit{cis},\textit{trans} methyl linolenate addition-elimination product. EI: M= 292 m/z (12\%), 263 m/z (7\%), 261 m/z (7\%), 108 m/z (44\%), 95 m/z (62 \%), 79 m/z (100 \%), 67 m/z (62 \%), 55 m/z (70 \%), 41 m/z (86 \%). Trans isomers have been reported to elute before \textit{cis} isomers on a polar phase cyano-polysiloxane column (84) like the one used in the analysis. Earlier, nitrogen dioxide was also reported by Khan to form \textit{cis},\textit{trans} isomers of methyl linolenate (86).

Reaction of Methyl Linolenate with 10 ppm of
Nitrogen Dioxide in an Ultra Pure Helium Carrier Gas (118): Methyl linolenate was purified according to the procedure given in chapter 2. The bubbler apparatus was assembled in the glove bag under an atmosphere of nitrogen. The nitrogen dioxide bulb was equilibrated for one hour after which a one hour blank was conducted. A Saltzman analysis (48) was performed on the 100 mL thymol trap solution to determine the concentration of nitrogen dioxide in the carrier gas. Methyl linolenate (1.4 mmoles) was placed in the bubbler and nitrogen dioxide in a helium carrier gas was bubbled through the methyl linolenate for one hour. The flow rate of the carrier gas was 60 mL/min and the temperature of the constant temperature bath was 37°C. The reaction mixture and starting material were analyzed by HPLC and GC analysis. Carvone and methyl linoelaidate were used as internal standards. Since both the hydroperoxides of methyl linolenate and the allylic nitrite(nitro) isomers decompose when concentrated, the response factor for methyl linoleate hydroperoxides was used to quantify the products.

Results

The allylic nitrite(nitro) isomers of methyl linolenate elute at similar retention times to the
Figure 18: HPLC chromatograms of methyl linolenate allylic nitrite(nitro) isomers (a) and methyl linolenate hydroperoxides (b).
hydroperoxides of methyl linolenate (Figure 18). The
elution of the allylic nitrite(nitro) isomers and
hydroperoxides of methyl linoleate were also shown to
have similar retention times (Chapter 3).

The HPLC chromatogram of the sodium borohydride
reduction products of the methyl linolenate
hydroperoxides are displayed in figure 17. The peak
assignments of the alcohols were obtained from methyl
linolenate alcohol assignments made by Chan and Levett
(83) under similar HPLC conditions. These methyl
linolenate alcohol assignments were used to determine
the identity of the unresolved hydroperoxides in each
peak in the chromatogram in figure 18. The UV maximum
of the hydroperoxide fractions and the hydroperoxide
assignments based on the sodium borohydride reduction
and GC-MS analysis are the following:

Table XIV
Hydroperoxide Fractions of Methyl Linolenate

<table>
<thead>
<tr>
<th>UV max</th>
<th>Hydroperoxide Fraction</th>
<th>Hydroperoxides</th>
</tr>
</thead>
<tbody>
<tr>
<td>236 nm</td>
<td>Fraction I</td>
<td>(13-c,t-RH)</td>
</tr>
<tr>
<td>234 nm</td>
<td>Fraction II</td>
<td>(12-c,t-RH), (12-t,t-RH),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16-c,t-RH)</td>
</tr>
<tr>
<td>234 nm</td>
<td>Fraction III</td>
<td>(13-t,t-RH), (16-t,t-RH)</td>
</tr>
<tr>
<td>234 nm</td>
<td>Fraction IV</td>
<td>(9-t,c-RH), (9-t,t-RH)</td>
</tr>
</tbody>
</table>
The proton NMR chemical shifts and splitting patterns of the olefinic region of the hydroperoxide fractions were similar to those of the allylic nitrite(nitro) isomers. The proton NMR's for fractions II and IV are presented below.

Table XV

<table>
<thead>
<tr>
<th></th>
<th>Fraction II</th>
<th>Fraction IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROOH</td>
<td>RNO2/RONO</td>
<td>ROOH</td>
</tr>
<tr>
<td>6.6 ppm (m)</td>
<td>6.6 ppm (m)</td>
<td>6.6 ppm (m)</td>
</tr>
<tr>
<td>6.05 ppm (t)</td>
<td>6.05 ppm (t)</td>
<td>6.05 ppm (t)</td>
</tr>
<tr>
<td>5.3-5.7 ppm</td>
<td>5.3-5.65 ppm</td>
<td>5.3-5.65 ppm</td>
</tr>
<tr>
<td>(br,m)</td>
<td>(br,m)</td>
<td>(br,m)</td>
</tr>
<tr>
<td>4.35 ppm(br,m)</td>
<td>4.35 ppm(m)</td>
<td>4.4 ppm (m)</td>
</tr>
</tbody>
</table>

The GC chromatograms in Figure 19 were used to distinguish the hydroperoxides from the allylic nitrite(nitro) isomers and to determine the positional
Figure 19: Gas chromatogram of methyl linolenate hydroperoxides and allylic nitrite(nitro) isomers. The arrows in figures a,b,e and f show the presence of methyl 13-oxo-9,11-tridecadienoate. The arrows in figures c and d show the presence of 2,4-heptadienal. The arrows in g and h show the presence of 2,4,7-decatrienal.
isomers present in each fraction. The positions of the functional groups were determined by identifying the aldehydes formed from carbon-carbon bond scission of the alkoxy radical intermediate of the hydroperoxides and allylic nitrite isomers. The aldehydes known to be formed from thermal homolysis of the hydroperoxides of methyl linolenate were reported by Frankel et al. (71). The same procedure was performed in chapter 3 with allylic nitriles and hydroperoxides of methyl linoleate.

The peaks at a retention time of 16.3 and 16.5 in the gc chromatogram of fraction I of the hydroperoxides and allylic nitrite isomers (Figures 19a & 19b) were tentatively identified as methyl 13-oxotridecadienoate originating from the 13-positional isomers (Scheme XI). (EI): M= 238 m/z (1.8%), [M-CH3OH]= 206 m/z (8.6%), [M-C2H2O2] = 178 m/z (8.4%).

The peak at a retention time of 5.5 in the gc chromatograms of fraction II hydroperoxides and allylic nitrite isomers (Figure 19c & 19d) was tentatively identified as 2,4-heptadienal originating from the 12-positional isomers (Scheme XII). (EI): M= 110 m/z (17%), [M-CHO] = 81 m/z (100%), [C4H7]= 55 m/z (7.1%). The aldehyde for the 16-allylic nitrite and 16-hydroperoxide (Scheme XIII) was not identified.
Scheme XI

The Formation of Methyl 13-oxo-9,11-Tridecadienoate from Thermal Homolysis of the 13-Hydroperoxide and 13-Allylic Nitrite Isomers of Methyl Linolenate

![Chemical Structure Diagram]
Scheme XII

The Formation of 2,4-Heptadienal from the Thermal Homolysis of the 12-Hydroperoxide and 12-Allylic Nitrite Isomers of Methyl Linolenate
Scheme XIII

The Formation of Methyl 16-Oxo-9,12,14-Hexadecatrienoate from the Thermal Homolysis of the 16-Hydroperoxide and 16-Allylic Nitrite Isomers of Methyl Linolenate
Scheme XIV

The Formation of 2,4,7-Decatrienal from the Thermal Homolysis of the 9-Hydroperoxide and 9-Allylic Nitrite Isomers of Methyl Linolenate
The peak at a retention time of 16.5 in the GC chromatograms of fraction III of the hydroperoxides and allylic nitrite isomers (Figure 19e & 19f) was previously tentatively identified as methyl 13-oxotridecadienoate arising from the 13-allylic nitrite and 13-hydroperoxide of methyl linolenate.

The peaks at a retention time of 10.1 and 10.5 in the GC chromatograms shown in figures 19g & 19h for fraction IV of the hydroperoxides and allylic nitrite isomers of methyl linolenate was tentatively identified as 2,4,7-decatrienal originating from the 9-positional isomers (Scheme XIV). (EI): M= 150 m/z (4.6%), [M-CHO]= 121 m/z (33%), [M-C5H9]= 81 m/z (100%), [C5H9]= 69 m/z (3.8%).

The negative methane chemical ionization spectra of the allylic nitrite (nitro) fractions are shown in figures 20-23. The allylic nitrite(nitro) isomers NCI spectra were also used to confirm the position of the nitrite functional group and the isomers present in each fraction. The molecular weight of the allylic nitrite(nitro) isomers was also determined. The same procedure was used in chapter 3 for the allylic nitrite(nitro) isomers of methyl linoleate. The NCI spectra of fraction I of the allylic nitrite(nitro) isomers is shown in Figure 20. The (M+H)- ion of 338
Figure 20: Negative chemical ionization spectrum of the allylic nitrite(nitro) isomers fraction I.
Figure 21: Negative chemical ionization spectrum of the allylic nitrite(nitro) isomers fraction II.
Figure 22: Negative chemical ionization spectrum of the allylic nitrite(nitro) isomers fraction III.
Figure 23: Negative chemical ionization spectrum of the allylic nitrite(nitro) isomers fraction IV.
m/z (20%) and M⁻ ion of 337 m/z (10%) were detected in this fraction confirming the molecular weight of the allylic nitrite(nitro) isomer. The 46 m/z (62%) indicative of a nitro functional group and the 62 m/z (5%) indicative of a nitrate functional was also detected. The 237 m/z ion is the (M-H)⁻ ion of methyl 13-oxotridecadienoate formed from O-N homolysis and subsequent carbon-carbon bond scission of the 13-allylic nitrite isomer (Scheme XI). The NCI spectrum of fraction II of the allylic nitrite(nitro) isomers is displayed in Figure 21. The (M+H)⁺ ion of 338 m/z, M⁻ ion of 337 m/z, NO₂⁻ ion of 46 m/z and the NO₃⁻ ion of 62 m/z can be seen in the spectrum. The 109 m/z (5%) ion is the (M-H)⁺ ion of 2,4-heptadienal formed from thermal homolysis of the 12-allylic nitrite isomer(s) (Scheme XII). The 277 m/z (38%) ion is the (M-H)⁻ ion of methyl 16-oxo-9,12,14-hexadecatrienoate isomer(s) formed from the thermal homolysis of the 16-allylic nitrite isomer (Scheme XIII). The 237 m/z ion for the (M-H)⁻ ion of methyl 13-oxotridecadienoate formed from the 13-allylic nitrite isomer was not detected.

The NCI spectrum of fraction III of the allylic nitrite(nitro) isomers is shown in figure 22. The (M+H)⁺ ion of 338 m/z (10%), M⁻ ion of 337 m/z (2%),
were all detected in the NCI spectra. The 237 m/z ion (7%) is the (M-$H$)$^-$ ion of methyl 13-oxo-9,11-
tridecadienoate formed from thermal homolysis of the 13-allylic nitrite isomer (Scheme XI). The 277 m/z (12%) ion is the (M-$H$)$^-$ ion of methyl 16-oxo-9,12,14-
hexadecatrienoate formed from the thermal homolysis of the 16-allylic nitrite isomer (Scheme XIII). The 109 m/z ion for the (M-$H$)$^-$ ion of 2,4-heptadienal formed from thermal homolysis of the 12-allylic nitrite isomer was not detected.

The NCI spectrum of fraction IV allylic nitrite(nitro) isomers is shown in Figure 23. The (M+$H$)$^+$ ion of 338 m/z, M$^-$ ion of 337 m/z, NO$_2^-$ ion of 46 m/z and the NO$_3^-$ ion of 62 m/z were all detected in the spectra. The 149 m/z ion (48%) is the (M-$H$)$^-$ ion of 2,4,7-decatrienial formed from thermal homolysis of the 9-allylic nitrite isomer (Scheme XIV). The 237 m/z ion for the (M-$H$)$^-$ ion of methyl 13-oxo-9,11-
tridecadienoate formed from thermal homolysis of the 13-allylic nitrite isomer, the 109 m/z ion for the (M-$H$)$^-$ ion of 2,4-heptadienal formed from the 12-allylic nitrite isomer, and the 277 m/z ion for the (M-$H$)$^-$ ion of methyl 16-oxo-9,12,14-hexadecatrienoate formed from the 16-allylic nitrite isomer were not detected in the
The assignments of the allylic nitrite(nitro) isomers present in the allylic nitrite(nitro) isomer fractions in the HPLC chromatogram shown in Figure 18 are displayed in Table XVI. The allylic nitrite(nitro) isomer assignments were obtained from the GC-MS and NCI data presented above. Since the geometrical isomers of the hydroperoxides and allylic nitrite(nitro) isomers of methyl linoleate elute at similar retention times by HPLC (chapter 3), the geometrical isomers present in the allylic nitrite(nitro) isomer fractions of methyl linolenate were assumed to be the same as the corresponding hydroperoxide isomer fractions that elute at similar HPLC retention times (Table XVI).

Presented in table XVII are the mole percentages of products detected in the nitrogen dioxide (10 ppm in helium) and methyl linolenate reaction mixture. The amount of allylic nitrite(nitro) H-abstraction products formed were $96.5 \pm 3.0\%$ with $3.5 \pm 0.3\%$ of the addition-elimination products detected. The products detected account for $98.1 \pm 11.2\%$ of the methyl linolenate reacted. The kinetic chain length of the reaction is $0.93 \pm 0.06$. The mole percentages of the products in the table are obtained from an
average of five HPLC analysis and four GC analysis.

Table XVI

Allylic Nitrite (Nitro) Isomers of Methyl Linolenate

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Allylic nitrite(nitro) isomers</th>
</tr>
</thead>
<tbody>
<tr>
<td>fraction I</td>
<td>(13-c,t-AN)</td>
</tr>
<tr>
<td>fraction II</td>
<td>(12-c,t-AN), (12-t,t-AN), and (16-c,t-AN)</td>
</tr>
<tr>
<td>fraction III</td>
<td>(13-t,t-AN) and (16-t,t-AN)</td>
</tr>
<tr>
<td>fraction IV</td>
<td>(9-c,t-AN) and (9-t,t-AN)</td>
</tr>
</tbody>
</table>
Table XVII

Mole Percentage of Methyl linolenate & NO2 Products

<table>
<thead>
<tr>
<th>Products</th>
<th>Mole percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion of Methyl Linolenate to Products: 2.0 ± 0.1%</td>
<td></td>
</tr>
</tbody>
</table>

Addition Products:

* cis,trans-18:3 * 3.50 ± 0.3%

H-abstraction Products:

* fraction I allylic nitrite(nitro) * 10.0 ± 1.5%
* fraction II allylic nitrite(nitro) * 53.1 ± 2.2%
* fraction III allylic nitrite(nitro) * 6.5 ± 0.3%
* fraction IV allylic nitrite(nitro) * 26.9 ± 1.1%

Discussion

The allylic nitrite(nitro) isomers of methyl linolenate are not resolved by adsorption phase HPLC. The hydroperoxides of methyl linolenate are also not resolved by adsorption phase HPLC. Despite the isomers of each type of compound not being resolved by HPLC, the isomers of the allylic nitrite(nitro) and
hydroperoxides compounds appear to elute at similar retention times by HPLC analysis.

Since the structures of the hydroperoxides are known (83), the spectral data of the hydroperoxides was used to elucidate the structures of the allylic nitrite isomers. The only difference between the allylic nitrite(nitro) isomers and the corresponding hydroperoxides is the functional group. The structures of the allylic nitrite(nitro) isomers being the same was demonstrated by the proton NMRs of fraction II and IV allylic nitrite(nitro) isomers and hydroperoxides compounds being similar (Table XV). The same trend in the proton NMRs of the allylic nitrite(nitro) compounds and hydroperoxides of methyl linolenate were seen for the allylic nitrite(nitro) compounds and hydroperoxides of methyl linoleate in chapter 3.

The hydroperoxides of methyl linolenate can undergo thermal homolysis of the O-O bond to form an alkoxy radical like the hydroperoxides of methyl linoleate. The homolysis of methyl linolenate hydroperoxides to form aldehydes and other compounds was demonstrated by Frankel et al. (71). The aldehydes produced from thermal homolysis of the hydroperoxide isomers were used to locate the position
of the hydroperoxy functional group. Since the allylic nitrite isomers can also form the intermediate alkoxy radical from the homolysis of the O-N bond, the aldehydes formed from homolysis of the hydroperoxides was also formed by the allylic nitrite isomers. These aldehydes were used to locate the position of the nitrite functional group and to determine the allylic nitrite(nitro) isomers present in the unresolved peaks. The aldehydes formed from thermal homolysis of the hydroperoxides and the allylic nitrite isomers were identified by GC-MS and NCI analysis.

The NCI analysis of the allylic nitrite(nitro) isomers confirmed the molecular weight of the compounds by the presence of a M⁻ ion of 337 m/z. An (M+H)⁺ ion was also seen for the allylic nitrite(nitro) compounds. The presence of a 46 m/z ion in the spectra demonstrated that the compounds contained a nitro functional group (76-78). The 62 m/z ion demonstrated the presence of a nitrate functional group (76-78). The nitrate functional group is believed to be formed from the termination of alkoxy radicals with NO2 in the source of the mass spectrometer. The alkoxy radicals are formed from the thermal homolysis of the allylic nitrite isomers.
Some of the nitrate or 62 m/z detected could also originate from the oxidation of the nitrite compounds (75).

The data above was used to identify the allylic nitrite(nitro) isomers formed from the reaction of nitrogen dioxide with methyl linolenate in the absence of oxygen. The identity of the allylic nitrite (nitro) isomers eluting by HPLC are given in table XVI. Similar to methyl linoleate, methyl linolenate allylic nitrite(nitro) isomers elute at similar retention times to the corresponding hydroperoxide isomers of methyl linolenate.

The results of the methyl linolenate reaction with 10 ppm of nitrogen dioxide in the absence of oxygen demonstrated that methyl linolenate can react with a low concentration of nitrogen dioxide primarily by a H-abstraction mechanism. This proves that low levels of nitrogen dioxide can only react with compounds with abstractable hydrogens by a H-abstraction mechanism.
CHAPTER 6
Nitrogen Dioxide Catalyzed Isomerization
of Methyl linoleate and Methyl linolenate
Hydroperoxides

Nitrogen dioxide has been shown to initiate autoxidation of polyunsaturated fatty acids by a H-atom abstraction mechanism. However, the hydroperoxides of methyl linoleate and methyl linolenate may also be able to react with a low concentration of nitrogen dioxide by a hydrogen abstraction mechanism.

An earlier study by Castle (30) demonstrated that NO$_2$/N$_2$O$_4$ reacted with the O-H bond of hydroperoxides and not by homolytic displacement of the O-O bond (31) of the hydroperoxides. If a low concentration of nitrogen dioxide can react with hydroperoxides by a H-abstraction mechanism, a peroxyl radical would be formed. In the case of methyl linoleate, the peroxyl radical can undergo isomerization to the other peroxyl radical isomers according to the Porter mechanism of hydroperoxide isomerization (Scheme III) (32). For the methyl linolenate hydroperoxides, the inner 13-hydroperoxide and 12-hydroperoxides can form hydroperoxy-cyclic peroxides (Scheme IV) (37). The
formation of hydroperoxy-cyclic peroxides can lead to malonaldehyde formation which is a compound that is known to cross-link proteins (34,35).

In order to determine whether the hydroperoxides of methyl linoleate and methyl linolenate can react with low levels of nitrogen dioxide by a H-abstraction mechanism, the hydroperoxides of methyl linoleate and some of the hydroperoxides of methyl linolenate were reacted with a low concentration of nitrogen dioxide.

Experimental

Chemicals:

Hydroperoxides of methyl linoleate and methyl linolenate were formed by a one week air oxidation of methyl linoleate and methyl linolenate. The hydroperoxides were separated and isolated according to the procedures given in chapter 3 & 5.

Dodecane (99+%, anhydrous under nitrogen, Aldrich) was used as the solvent for the reactions. Instrumentation: The instrumentation and conditions are given in chapter 3.

Procedures:

The following molar concentrations of
hydroperoxides of methyl linoleate in dodecane were reacted with nitrogen dioxide in helium or air according to the procedures given in chapter 2 (119):

A. 13-c,t-hydroperoxide (5.3 mM); 4 ppm of NO₂ in He
B. 13-c,t-hydroperoxide (2.9 mM); 3.0 ppm of NO₂ in air.
C. 13-c,t-hydroperoxide (2.2 mM); air
D. 13-t,t-hydroperoxide (2.6 mM); < 3.0 ppm of NO₂ in He.
E. 9-t,t-hydroperoxide (2.2 mM); 3.0 ppm of NO₂ in He.

Methyl linolenate hydroperoxide peak fractions II and IV were isolated and reacted with 3 ppm of nitrogen dioxide in air to determine if hydroperoxy-cyclic peroxides would be formed (120). The procedure is given in chapter 2.

Product analysis:

The cyclic peroxide formed from methyl linolenate fraction II reacting with nitrogen dioxide in air was tentatively identified from an air oxidized sample of methyl linolenate that eluted at a similar HPLC retention time. The air oxidized hydroperoxy-cyclic peroxide was analyzed by NMR and GC-MS analysis (121).
The proton NMR spectrum appeared to be a mixture of cyclic peroxides formed from the 13 and 12 hydroperoxides of methyl linolenate. The chemical shifts were matched to published chemical shifts of cyclic peroxides (87) formed from the 13 and 12 hydroperoxides of linolenate.

The GC chromatogram of the cyclic peroxide (Figure 24) have peaks at a retention time of 16.3 and 16.5. These peaks have the same retention time and mass spectrum as the peaks assigned as methyl 13-oxotridecadienoate in the gc chromatogram of the methyl linolenate hydroperoxide fraction I shown in figure 19a. Methyl 13-oxotridecadienoate is a known thermal decomposition product of methyl 16-hydroperoxy-13,15-epidioxy-9,11-octadecadienoate formed from the 13-hydroperoxide of methyl linolenate (88). The peak at a retention time of 5.5 in the gc chromatogram of the cyclic hydroperoxides have the same retention time and mass spectrum as the peak assigned as 2,4-heptadienal in the gc chromatogram of the methyl linolenate hydroperoxide fraction II (Figure 19c). 2,4-Heptadienal is another known thermal decomposition product of methyl 9-hydroperoxy-10,12-epidioxy-13,15-octadecadienoate formed from the 12-hydroperoxide of methyl linolenate (88).
Table XVIII
Proton NMR Data of Cyclic Peroxides

<table>
<thead>
<tr>
<th>Isolated cyclic ROOH</th>
<th>Published (87) Values of cyclic peroxides formed from 13-ROOH</th>
<th>12-ROOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical shift/ mult./ # H</td>
<td>Chemical shifts</td>
<td></td>
</tr>
<tr>
<td>9.5 ppm (s)</td>
<td>---</td>
<td>9.52 ppm</td>
</tr>
<tr>
<td>6.65 ppm (t)</td>
<td>---</td>
<td>6.67 ppm</td>
</tr>
<tr>
<td>6.0 ppm (m)</td>
<td>---</td>
<td>6.00 ppm</td>
</tr>
<tr>
<td>5.55 ppm (m)</td>
<td>1 H</td>
<td>5.62 ppm</td>
</tr>
<tr>
<td>5.35 ppm (m)</td>
<td>2 H</td>
<td>5.54 ppm</td>
</tr>
<tr>
<td>4.85 ppm (m)</td>
<td>---</td>
<td>4.80 ppm</td>
</tr>
<tr>
<td>4.50 ppm (m)</td>
<td>---</td>
<td>4.49 ppm</td>
</tr>
<tr>
<td>4.35 ppm (m)</td>
<td>---</td>
<td>4.14 ppm</td>
</tr>
<tr>
<td>4.15 ppm (m)</td>
<td>---</td>
<td>2.82 ppm</td>
</tr>
<tr>
<td>2.80 ppm (m)</td>
<td>2H</td>
<td></td>
</tr>
</tbody>
</table>

The peak at a retention time of 16.3 and 5.5 originates from O-O homolysis of the cyclic peroxide ring to form an alkoxy radical (88). The homolysis of the cyclic peroxide ring was reported by Frankel et al. in the GC analysis of linolenate cyclic peroxides.
Figure 24: Gas chromatograms of the cyclic peroxide (a) and methyl linolenate hydroperoxide fraction I (b) and II (c). The arrows in (a) and (b) show the formation of methyl 13-oxo-9,11-tridecadienoate. The arrows in (a) and (c) show the formation of 2,4-heptadienal.
(88). The alkoxyl radical can undergo carbon-carbon bond scission to form aldehydes that can be used to locate the position of the ring. The peak at a retention time of 16.3 indicates the presence of the 16-hydroperoxy-13,15-peroxy-9,11-octadecadienoate obtained from the cyclic peroxide formation of the 13-hydroperoxide of methyl linolenate. The peak at a retention time of 5.5 indicates the presence of 9-hydroperoxy-10,12-peroxy-13,15-octadecadienoate obtained from the cyclic peroxide formation of the 12-hydroperoxide of methyl linolenate.

Results

A table of the isomers formed when the hydroperoxides of methyl linoleate are exposed to nitrogen dioxide in helium are shown below. The (+) sign designates the formation of the isomer and the (-) sign designates that the isomer was not detected.
Table XIX
Hydroperoxide Reaction with Nitrogen Dioxide

<table>
<thead>
<tr>
<th>Isomers:</th>
<th>13-c,t</th>
<th>13-t,t</th>
<th>9-c,t</th>
<th>9-t,t</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-c,t</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>13-t,t</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9-t,t</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The results above appear to demonstrate that nitrogen dioxide may react by an addition-elimination mechanism instead of a H-abstraction mechanism. Nitrogen dioxide reacting by an addition-elimination mechanism with conjugated double bonds was reported earlier by Ohta et al. (62). If nitrogen dioxide reacted by a H-abstraction mechanism, the 13-c,t-hydroperoxide would have formed the 9-t,t isomer, according to the Porter mechanism of hydroperoxide isomerization (32), instead of the 13-t,t isomer that was formed. The 13-t,t-hydroperoxide, in accordance with the Porter mechanism (32), would have formed the 9-t,t isomer and the 9-t,t-hydroperoxide would have formed the 13-t,t isomer.

However, when the 13-c,t-hydroperoxide was reacted with nitrogen dioxide in air, the
hydroperoxide isomerized to the other three isomers. The 13-c,t-hydroperoxide when bubbled with only air for one hour did not isomerize. Apparently, nitrogen dioxide can catalyze the isomerization of the hydroperoxides but the catalysis of isomerization is not initiated by the hydroperoxide reacting with nitrogen dioxide by a H-abstraction mechanism.

Nitrogen dioxide appears to be reacting with the hydroperoxide by an addition-elimination reaction. Whether nitrogen dioxide catalyzes the isomerization of the hydroperoxides by adding to the double bond is not known because a complete product study was not performed. Nitrogen dioxide may also react by a homolytic displacement of the O-O bonds of the hydroperoxides forming an alkoxy radical that could abstract a hydrogen atom catalyzing the isomerization of the hydroperoxides. Nitrogen dioxide reacting by a homolytic displacement of the O-O bonds was the mechanism proposed for the gas phase reaction of nitrogen dioxide with hydrogen peroxide (31).

Since nitrogen dioxide can catalyze the isomerization of methyl linoleate hydroperoxides, nitrogen dioxide may also be able to catalyze the formation of hydroperoxy-cyclic peroxides. The cyclic peroxides of methyl linolenate are only formed from
Figure 25: HPLC chromatograms of methyl linolenate hydroperoxide fraction II (a) and IV (b) reaction with nitrogen dioxide in air. The arrow in (a) shows the formation of the cyclic peroxide.
the inner 13-hydroperoxide and 12-hydroperoxide (37). To determine whether cyclic peroxides could be formed, the methyl linolenate hydroperoxide fractions II and IV (Chapter 5) were reacted with nitrogen dioxide in air. Methyl linolenate hydroperoxide fraction II contains the 12-hydroperoxides and the 16-hydroperoxide while fraction IV contains only the 9-hydroperoxides of methyl linolenate. Methyl linolenate fraction II formed a trace amount of the hydroperoxy-cyclic peroxide but the methyl linolenate hydroperoxide fraction IV did not (Figure 25).

Discussion

Nitrogen dioxide can catalyze the isomerization of the hydroperoxides of methyl linoleate and cyclic peroxide formation of the inner hydroperoxides of methyl linolenate. But the isomerization and cyclic peroxide formation of the hydroperoxides is not initiated by nitrogen dioxide reacting with the hydroperoxides by a H-abstraction mechanism. Hydrogen atom abstraction by nitrogen dioxide was demonstrated not to occur when the hydroperoxides of methyl linoleate were reacted with nitrogen dioxide in the absence of oxygen. However, nitrogen dioxide did
appear to react by an addition-elimination mechanism. Apparently, hydroperoxides do not have hydrogen atoms abstractable enough to react with nitrogen dioxide by a H-abstraction mechanisms.

Methyl oleate was also demonstrated to react by an addition mechanism instead of a H-abstraction mechanism (Chapter 1). Nitrogen dioxide not being able to react by a H-abstraction mechanism with hydroperoxides and methyl oleate can be explained by the change in the Gibbs free energy for H-abstraction. The change in the Gibbs free energy for H-abstraction for methyl oleate and the hydroperoxides can be calculated using the following equation (67):

$$\Delta G = -23.06 \Delta E$$

and the reduction potentials for nitrogen dioxide of 1.04 eV (66), methyl oleate 0.96 ± 0.06 eV (from H-allylic of propene) (67), hydroperoxides of 1.0 ± 0.06 eV (68), and methyl linoleate & methyl linolenate of 0.60 ± 0.06 eV (from H-pentadienyl-1,4-yl-3) (67).

Methyl oleate and the hydroperoxides have a Gibbs free energy change for reacting with nitrogen dioxide by a H-abstraction mechanism of -1.8 ± 1.8 kcal/mole and -0.9 ± 2.0 kcal/mole or approximately zero. However, methyl linoleate and methyl linolenate have a Gibbs free energy change for reacting with nitrogen
dioxide by a H-abstraction mechanism of \(-10.1 \pm 1.8\) kcal/mole. Therefore, nitrogen dioxide reacting with methyl linoleate and methyl linolenate by a H-abstraction mechanism is favored; whereas nitrogen dioxide reacting with methyl oleate and hydroperoxides of methyl linoleate and methyl linolenate by a H-abstraction mechanism is not favored.

Since nitrogen dioxide does not react with hydroperoxides by a H-abstraction mechanism, nitrogen dioxide must initiate the isomerization of methyl linoleate hydroperoxides and the formation of methyl linolenate hydroperoxy-cyclic peroxides by either an addition mechanism or homolytic cleavage of the O-O bond to form an alkoxy radical.
CHAPTER 7
The Preventative Antioxidant Abilities
of
Vitamin E and Vitamin C

Low concentrations of nitrogen dioxide have been shown to react with polyunsaturated fatty acids like methyl linoleate and methyl linolenate by a H-abstraction mechanism to initiate autoxidation. When a 50:50 molar solution of methyl oleate and methyl linoleate reacts with a low concentration of nitrogen dioxide, H-abstraction is the only mechanism that occurs. Therefore, low concentrations of nitrogen dioxide will probably react with polyunsaturated fatty acid components of phospholipids that comprise cell membranes exclusively by a H-abstraction mechanism.

Low levels of nitrogen dioxide have also been linked to the spread of cancer (4-7). The spread of cancer is believed to be caused in part by a reduction of the number of immune cells in animals upon inhalation of nitrogen dioxide (4-7). If H-abstraction occurs in the cell membrane, nitrous acid will be formed directly in the membrane(19). This nitrous acid can nitrosate amide linkages forming potent carcinogenic nitrosamides(25,26). Studies have
shown that nitrosamine formation in animals can occur upon animal exposure to low levels of nitrogen dioxide (27). The possible formation of potent carcinogenic nitrosamides may be another reason low concentrations of nitrogen dioxide can cause cancer to spread.

In order to prevent low concentrations of nitrogen dioxide from initiating autoxidation of polyunsaturated fatty acids and forming nitrous acid in the interior of the membrane, ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) (Figure 26) were studied to determine if these compounds could act as preventative antioxidants.

Vitamin C is a water soluble antioxidant that can reduce alpha-tocopherol radicals to alpha tocopherol by donating a hydrogen atom to the tocopherol radical (40-42). Vitamin C can also inhibit both in vivo and in vitro nitrosation of amines (89). The inhibition of nitrosation by vitamin C is believed to occur by vitamin C reducing nitrous acid to nitric oxide (90). Vitamin C can also act as a weak chain breaking antioxidant (61).

Vitamin E is a lipid soluble antioxidant that is believed to be oriented in the membrane with the phenolic head group near the membrane surface and the
Figure 26: Vitamin C and Vitamin E.
hydrophobic tail embedded in the interior of the membrane (91). Alpha-tocopherol can also inhibit N-nitrosation in lipids by reducing nitrite to nitric oxide (90). Vitamin E acts as a chain breaking antioxidant in the inhibition of autoxidation of unsaturated fatty acids (60,92). The chain breaking antioxidant activity of vitamin E occurs by alpha-tocopherol donating a hydrogen atom to peroxyl radicals preventing the unsaturated fatty acid substrate from being oxidized, via a H-abstraction mechanism, by the peroxyl radical (92).

In order to determine if vitamin E or vitamin C can prevent a low concentration of nitrogen dioxide from reacting with methyl linoleate, nitrogen dioxide in the absence of oxygen was reacted with methyl linoleate in an aqueous buffered system in the presence and absence of vitamin C and vitamin E.

Experimental

Materials:

Ascorbic acid (Aldrich), methyl linoleate (99% by GC, Sigma) purified according to the procedure given in chapter 2, and d,l-alpha-tocopherol (99%, Sigma). The other materials and equipment used are given in
chapter 2.

Procedure:

Buffered Aqueous Reaction (122): A buffered solution (oxygen free) was made according to the procedures given in chapter 2. The bubbler apparatus was assembled in a glovebag under an atmosphere of nitrogen. The temperature of the constant temperature bath was 37°C. The flow rate of the ultra pure helium carrier gas was 60 mL/min. The nitrogen dioxide bulb was opened and equilibrated for one hour. A 1L 0.1 N sodium hydroxide and thymol trap was used to trap the nitrogen dioxide. A one hour blank was conducted to determine the nitrogen dioxide in helium concentration. The sodium phosphate buffer (2 mL) was added to the bubbler and 47 ppm of nitrogen dioxide was bubbled through the buffer for one hour. The bubbler was rinsed three times with buffer and 2 mL of buffer was placed in the bubbler and methyl linoleate (1.3 mmoles) was placed on the surface of the buffer. Nitrogen dioxide in helium was bubbled through the mixture for one hour. The methyl linoleate was extracted from the aqueous layer with dichloromethane (oxygen free), dried with magnesium sulfate, filtered and concentrated. The methyl linoleate reaction
mixture and starting material were analyzed by HPLC and GC analysis. A Saltzman analysis was performed on the thymol trap used for the one hour blank, the buffer blank and the reaction.

Buffered Aqueous Vitamin C Reaction (123): A pH 7.4, 0.5 M sodium phosphate buffer (oxygen free) was used to make a $4.9 \times 10^{-4}$ M vitamin C solution. The reaction was conducted according to the procedures given above. However, a buffer blank and vitamin C containing buffer solution blank were conducted. The nitrogen dioxide concentration in ultra pure helium was 43 ppm and 1.1 mmole or 3.0 M methyl linoleate was used for the reaction. The amount of vitamin C reacted was determined by UV analysis of the unreacted vitamin C solution and the vitamin C solution blank at a wavelength maximum of 262 nm and 8100 cm$^{-1}$ M$^{-1}$ molar absorptivity in a pH 7.4, 0.5M sodium phosphate buffer.

Aqueous Vitamin E Reaction (124): Alpha-tocopherol (0.46 mM or 0.02 %wt) in neat methyl linoleate was used for the reaction. The reaction was conducted according to the procedures given above. Nitrogen dioxide in helium (48 ppm) and 1.3 mmole or 3.0 M methyl linoleate containing vitamin E was used
for the reaction.

Aqueous Vitamin C and Vitamin E Reaction (125):
A 0.53 mM (0.02 % wt) alpha-tocopherol in methyl linoleate solution and a 0.51 mM vitamin C in 0.5M, pH 7.4 sodium phosphate buffer (oxygen free) were made. The reaction was conducted according to the above procedures. Nitrogen dioxide in helium (45 ppm) and 1 mmole or 3.0 M methyl linoleate containing vitamin E was used for the reaction.

Products: Identified in chapter 2 and 3.

Results
The moles of products formed from the 47 ppm of nitrogen dioxide and neat methyl linoleate reaction is shown in Table XX. The four allylic nitro(alcohol) isomer H-abstraction products from the hydrolysis of the allylic nitrite derivative account for 89 ± 2.6 mole % of the products formed. The conversion of methyl linoleate to products was 14 ± 1.4 %.
Table XX

Moles of Products Formed from Nitrogen Dioxide Reacting with Methyl Linoleate in the Presence and Absence of Vitamin C and Vitamin E in Helium at 37°C

<table>
<thead>
<tr>
<th>ppm of NO₂:</th>
<th>47 ± 3</th>
<th>43 ± 2</th>
<th>48 ± 3</th>
<th>45 ± 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Conversion 18:2=</td>
<td>14</td>
<td>11</td>
<td>3.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Tot. add. prod.=</td>
<td>0.20</td>
<td>---</td>
<td>0.22</td>
<td>0.015</td>
</tr>
<tr>
<td>Tot. H-abstr.=</td>
<td>1.6</td>
<td>1.2</td>
<td>0.25</td>
<td>0.016</td>
</tr>
<tr>
<td>% Con.Vit. E=</td>
<td>---</td>
<td>---</td>
<td>86</td>
<td>75</td>
</tr>
<tr>
<td>% Con.Vit. C=</td>
<td>---</td>
<td>1.6</td>
<td>---</td>
<td>0.10</td>
</tr>
<tr>
<td>% NO₂ used=</td>
<td>38</td>
<td>52</td>
<td>40</td>
<td>53</td>
</tr>
</tbody>
</table>

The uncertainty in the moles of products range from 0.001-0.05.

The moles of products formed from the 43 ppm of nitrogen dioxide and methyl linoleate reaction in the presence of vitamin C is also shown in Table XX. The allylic nitro(alcohol) isomers were still formed in the presence of vitamin C. The conversion of methyl
linoleate to products was $11 \pm 0.07$ mole %. The amount of vitamin C reacted was 1.6 mole %.

The moles of products formed from reacting 48 ppm of nitrogen dioxide with methyl linoleate and vitamin E are displayed in Table XX. A small amount of the allylic nitro(alcohol) isomer are formed. The mole percentage of H-abstraction is $53 \pm 3.5$ % with the conversion to products being only $3.6 \pm 0.07$ %. The moles of addition products formed remain practically the same. The vitamin E had a conversion to products of 86 mole %.

The moles of products formed from the 45 ppm of nitrogen dioxide and methyl linoleate reaction in the presence of vitamin C and vitamin E are shown in Table XX. The H-abstraction products formed account for $53 \pm 2.2$ % of the products. The conversion of methyl linoleate to products is $3.1 \pm 0.07$ %. The conversion of vitamin E to products was $75 \pm 7.1$ % with the conversion of vitamin C being only 0.1%. The addition products were still formed accounting for $47 \pm 0.9$ mole% of the products detected.
Discussion

Vitamin E prevented nitrogen dioxide from reacting with methyl linoleate by a H-abstraction mechanism but not by an addition mechanism. Vitamin C does not appear to be able to prevent nitrogen dioxide from reacting with methyl linoleate by a H-abstraction mechanism. However, vitamin C in the presence of vitamin E can reduce the conversion of vitamin E and still prevent nitrogen dioxide from reacting by a H-abstraction mechanism.

Vitamin E appears to be able to act as a preventative antioxidant in the presence of a low concentration of nitrogen dioxide by donating a hydrogen atom to nitrogen dioxide. Therefore, at environmentally relevant concentrations of nitrogen dioxide in which nitrogen dioxide would initiate autoxidation of unsaturated fatty acids by a H-abstraction mechanism, vitamin E should be able to prevent nitrogen dioxide from coming in contact with the unsaturated fatty acid.

Although vitamin E reacts with nitrogen dioxide by a H-abstraction mechanism to form nitrous acid, nitrous acid will not be formed in the interior region of the membrane. The phenolic O-H group is the
reactive site of vitamin E (91). This phenolic head group is situated near the surface of the membrane (91). Therefore when nitrous acid is formed on the surface of the membrane vitamin C can reduce the nitrous acid to nitric oxide (90).

According to the results, vitamin C can not donate a hydrogen atom to nitrogen dioxide in order to prevent nitrogen dioxide from reacting with methyl linoleate by a H-abstraction mechanism. However, vitamin C can decrease the amount of vitamin E converted to products. Vitamin C may be protecting vitamin E by reducing the tocopheroyl radical formed from H-abstraction to tocopherol (40-42). The reduction of vitamin E by vitamin C may not be occurring by a H-atom donation but by an electron transfer mechanism to form an anion with subsequent proton transfer to the anion. The electron transfer mechanism seems plausible since vitamin C is known to reduce Fe$^{3+}$ to Fe$^{2+}$ (93).

Although low concentrations of nitrogen dioxide have been shown to react with polyunsaturated fatty acids by a H-abstraction mechanism, vitamin E can prevent the reaction from occurring. Since nitrogen dioxide initiated autoxidation by a H-abstraction mechanism will not occur in the presence of vitamin E,
nitrous acid and therefore nitrosamides should not be formed in the cell membranes of animals on a diet rich in vitamin E when the animals are exposed to low levels of nitrogen dioxide. Therefore, vitamin E can be used to inhibit autoxidation, to prevent low levels of nitrogen dioxide from initiating autoxidation and to prevent the formation of potent carcinogenic nitrosamides in the cell membrane.
Low concentrations of nitrogen dioxide have been shown to react with polyunsaturated fatty acid esters primarily by a H-atom abstraction mechanism. Although methyl oleate, an unsaturated fatty acid, does not react by a H-abstraction mechanism, a 50:50 molar solution of methyl oleate and methyl linoleate reacts with a low concentration of nitrogen dioxide exclusively by a H-atom abstraction mechanism.

When methyl linoleate is diluted with methyl palmitate in the anaerobic reactions, a decrease in the mole percentage of conversion of methyl linoleate to products is detected (Chapter 2, Table IV). The decrease in the conversion of methyl linoleate to products in the anaerobic reactions suggests that methyl palmitate decreases the probability of nitrogen dioxide reacting with methyl linoleate. Palmitic acid, linoleic acid and arachadonic acid increase in concentration in pulmonary phospholipids upon exposure of animals to low concentrations of nitrogen dioxide; however, oleic acid decreases in concentration in the lipids (43,44). The increase in the cellular synthesis of the polyunsaturated fatty acids is
probably caused by nitrogen dioxide attacking linoleic and arachadonic acid instead of oleic acid by a H-abstraction mechanism. The increase in the cellular synthesis of palmitic acid is probably done to decrease the probability of nitrogen dioxide reacting with the polyunsaturated fatty acid constituents.

The hydroperoxides of methyl linoleate also do not react with nitrogen dioxide by a H-abstraction mechanism. Despite low concentrations of nitrogen dioxide not reacting with hydroperoxides of methyl linoleate and methyl linolenate by a H-abstraction mechanism, nitrogen dioxide can still catalyze the isomerization and cyclic-peroxide formation of the hydroperoxides. The catalyzed isomerization and cyclic-peroxide formation of the hydroperoxide by nitrogen dioxide is believed to be caused by either nitrogen dioxide reacting by homolytic bond scission of the O-O bonds or by an addition mechanism.

The reason given for polyunsaturated fatty acids reacting with nitrogen dioxide by a H-abstraction mechanism and methyl oleate and the hydroperoxides of methyl linoleate not reacting by a H-abstraction mechanism is the following: The change in the Gibbs free energy for H-abstraction for methyl oleate and the hydroperoxides with nitrogen dioxide is
approximately zero whereas the change in the Gibbs free energy for H-abstraction for the polyunsaturated fatty acids like methyl linoleate and methyl linolenate with nitrogen dioxide is $-10.1 \pm 1.8$ kcal/mole. Therefore, methyl linoleate and methyl linolenate will react more spontaneously with nitrogen dioxide by a H-abstraction mechanism than methyl oleate and the hydroperoxides.

Since low levels of nitrogen dioxide can react with polyunsaturated fatty acids by a H-abstraction mechanism, nitrous acid can be formed in the cell membrane of animals exposed to low concentrations of nitrogen dioxide. The formation of nitrous acid directly in the membrane can cause the formation of potent carcinogenic nitrosamides (25,26). The production of nitrosamides in the membrane may be another reason low levels of nitrogen dioxide can cause cancer to spread in animals.

However, vitamin E can be used to prevent low levels of nitrogen dioxide from reacting with polyunsaturated fatty acids in the membrane and to prevent the formation of potent carcinogenic nitrosamides in the interior of the membrane. Although low levels of nitrogen dioxide can initiate autoxidation of polyunsaturated fatty acids by a H-
abstraction mechanism, the reaction can be prevented by vitamin E.
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VITA

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Major Field: Chemistry

Title of Dissertation: The Mechanism of Low Levels of Nitrogen Dioxide Reaction with Unsaturated Fatty Acid Esters

Approved:

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Major Professor and Chairman

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Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

November 29, 1990