The Identification of the Allylic Nitrite and Nitro Derivatives of Methyl Linoleate and Methyl Linolenate by Negative Chemical Ionization Mass Spectroscopy

Aris A. Gallon and **William A. Pryor***
Chemistry Department and the Biodynamics Institute, Louisiana State University, Baton Rouge, Louisiana 70803

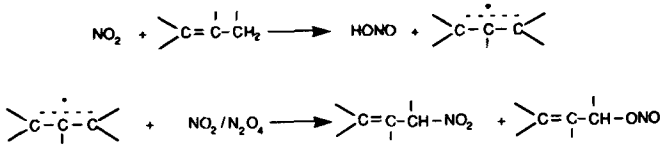
The autoxidation of polyunsaturated fatty acids is initiated both *in vivo* and *in vitro* by nitrogen dioxide. The mechanism of the initiation process is believed to involve both addition reactions and hydrogen atom abstraction reactions, with hydrogen abstraction predominating at low levels of nitrogen dioxide. Therefore low levels of nitrogen dioxide should react with polyunsaturated fatty acids to give allylic derivatives; in an anaerobic system these derivations should be allylic nitro and nitrite compounds. Using negative methane chemical ionization mass spectrometry and other analytical techniques, we have identified these allylic nitrite and nitro compounds from the reactions of low levels of nitrogen dioxide with methyl linoleate and methyl linolenate in the absence of oxygen. *Lipids 28,* **125-133 (1993).**

Nitrogen dioxide, a toxin present in polluted air (1,2), has been reported to cause pulmonary edema, pulmonary fibrosis, bronchitis (1,2) and cancer (3-6). Low levels of nitrogen dioxide have been shown to cause the initiation of the autoxidation of polyunsaturated fatty acids both *in vivo* (7-11) and *in vitro* (10,12). Nitrogen dioxide-initiated autoxidation of polyunsaturated fatty acids in cell membranes can lead to membrane damage and eventually cell death (7-10,13).

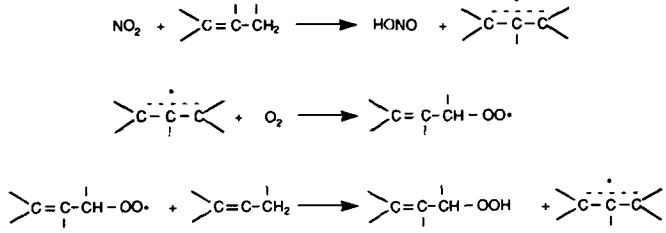
We previously reported that low levels of nitrogen dioxide initiate the autoxidation of cyclohexene by a hydrogen-atom abstraction mechanism (14,15). Recently, Postlethwait and Bidani (11) have demonstrated that nitrogen dioxide in an isolated rat lung reacts by a H-atom abstraction mechanism.

In the H-abstraction mechanism (Schemes 1 and 2), nitrogen dioxide abstracts a hydrogen atom from the allylic position of an alkene forming a resonance-stabilized radical. In the absence of oxygen (Scheme 1), nitrogen dioxide combines with the resonance-stabilized radical forming an allylic nitro or nitrite compound; in the presence of oxygen (Scheme 2), the resonance-stabilized radical combines with oxygen forming a peroxyl radical that ultimately forms allylic hydroperoxides (14). Since allylic compounds are formed *via* a H-atom abstraction mechanism, the detection of these allylic nitro or nitrite compounds can be used as a marker for nitrogen dioxide-induced H-abstraction.

In this study, we used negative chemical ionization to identify the allylic nitrite and nitro compounds formed by the reaction of nitrogen dioxide with methyl linoleate and methyl linolenate in the absence of oxygen. The allylic nitrite and nitro isomers identified are similar to the geometrical and positional isomers of methyl linoleate and methyl linolenate hydroperoxides formed in autoxidation reactions (16). Analogous to the hydroperoxide isomers, not all possible diasteriomers are produced (17). The following system is used to abbreviate the allylic nitrite (nitro) and hydroperoxide isomers: The abbreviations for two typical compounds are shown in Figure 1. The letters *c* or *t* denote a *cis* or *trans* double bond. The position of the double bond is placed before the letter *c* or *t*. The



SCHEME 1



SCHEME 2

*To whom correspondence should be addressed at Biodynamics Institute, 711 Choppin Hall, Louisiana State University, Baton Rouge, LA 70803-1804.

Abbreviations: AN, allylic nitro or nitrite compounds; *c*, *cis* double bond; DTPA, diethylenetriaminepentaacetic acid; GC, gas chromatography; HPLC, high-performance liquid chromatography; IR, infrared; M^-, parent ion; *m/z*, mass/charge; NCI, negative chemical ionization; NMR, nuclear magnetic resonance; HP, hydroperoxide; *t*, *trans* double bond; TLC, thin-layer chromatography; UV, ultraviolet; 9*c*,11*t*,15*c*-13AN, methyl 13-nitrito-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate and methyl 13-nitro-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate; 9*t*,11*t*,15*c*-13AN, methyl 13-nitrito-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate and methyl 13-nitro-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate; 9*c*,11*t*,15*c*-13HP, methyl 13-hydrperoxy-*cis*-9,*trans*-11*cis*-15-octadecatrienoate; 9*t*,11t,15*c*-13HP, methyl 13-hydroperoxy-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate; 9*c*,12*c*,14*t*-16AN, methyl 16-nitrito-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate and methyl 16-nitro-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate; 9*c*,12*t*,14t-16AN, methyl 16-nitrito-*cis*-9,*trans*-12,*trans*-14-octadecatrienoate and methyl 16-nitro-*cis*-9,*trans*-12,*trans*-14-octadecadienoate; 9*c*,12*c*,14*t*-16HP, methyl 16-hydroperoxy-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate; 9*c*,12*t*,14*t*-16HP, methyl 16-hydroperoxy-*cis*-9,*trans*-12,*trans*-14-octadecatrienoate; 9*c*,13*t*,15*c*-12AN, methyl 12-nitrito-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate and methyl 12-nitro-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate; 9*c*,13*t*,15*t*-12AN, methyl 12-nitrito-*cis*-9,*trans*-13,*trans*-15-octadecatrienoate and methyl 12-nitro-*cis*-9,*trans*-12,*trans*-15-octadecatrienoate; 9*c*,13t,15*c*-12HP, methyl 12-hydroperoxy-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate; 9*c*,13*t*,15*t*-12HP, methyl 12-hydroperoxy-*cis*-9,*trans*-13,*trans*-15-octadecatrienoate 10*t*,12*c*,15*c*-9AN, methyl 9-nitrito-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate and methyl 9-nitro-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate; 10*t*,12t,15*c*-9AN, methyl 9-nitrito-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate and methyl 9-nitro-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate; 10*t*,12*c*,15*c*-9HP, methyl 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate; 10*t*,12*t*,15*c*-9HP, methyl 9-hydroperoxy-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate.

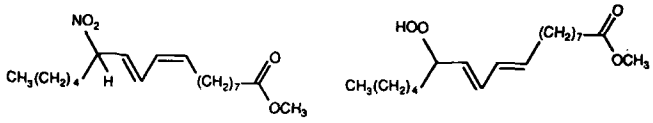


FIG. 1. Examples of the abbreviation systems used: Methyl 13-nitro-*cis*-9,*trans*-11-octadecadienoate and methyl 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate are abbreviated as 9*c*,11*t*-13AN and 9*c*,11*t*-13HP.

letters "AN" are used to signify an allylic nitro or nitrite group and the letters "HP" denote a hydroperoxide functional group. The position of the functional group is placed before the letters AN or HP.

EXPERIMENTAL PROCEDURES

Materials. Methyl linoleate and methyl linolenate, 99% pure by gas chromatography (GC) (Sigma, St. Louis, MO), was purified by eluting the esters through four columns each of which contained 1.0 g of alumina (neutral, Aldrich, Milwaukee, WI), and the last of which also contained in the tip 0.02 g of diethylenetriaminepentaacetic acid (DTPA) to remove any trace metals. These purification steps were done in a glove bag under nitrogen.

Nitrogen dioxide was generated from dinitrogen tetroxide (99.5%, Matheson, Secaucus, NJ). Dinitrogen tetroxide was placed in a glass bulb with phosphorus pentoxide and purged of oxygen with argon by freezing and thawing the dinitrogen tetroxide three times. Isopentyl nitrite (97%, Aldrich), and nitrocyclohexane (97%, Aldrich) were used as infrared (IR) standards. Hexane, isopropanol and dichloromethane, all high-performance liquid chromatography (HPLC) grade (Mallinckrodt, St. Louis, MO), were purged of oxygen before use. Ultra pure helium (99.9999%, chromatographic grade, Air Products, Allentown, PA) was used as the carrier gas for the reaction. A thymol trap was made by dissolving 0.4 g of thymol (Sigma) and 4 g of sodium hydroxide (97%, EM Science, Gibbstown, NJ) in 1 L of deionized water.

Instrumentation. The Ultraviolet (UV) spectra were taken on a Hewlett-Packard (Avondale, PA) 8451A diode array spectrophotometer in hexane/isopropanol (99:1, vol/vol); the IR spectra were obtained on an IBM (Armonk, NY) IR/45 spectrometer. HPLC was done on a Varian (Palo Alto, CA) 5000 series instrument with a UV detector set at a 215 nm wavelength with a 16-nm slit width was used to separate the hydroperoxide and allylic nitro and nitrite isomers. A 25 × 0.46 cm silica column (Rainin, Woburn, MA; Microsorb) was used with hexane/isopropanol (99:1, vol/vol) at a flow rate of 1 mL/min. The nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (Billerica, MA) 200 MHz instrument using deuterated chloroform as the solvent. Negative methane chemical ionization mass spectrometry was performed on a Finnigan MAT (San Jose, CA) TSQ 4500 mass spectrometer using a direct exposure probe ramped to 1 A at a rate of 10 mA/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 amu. The source temperature was 150°C and the manifold temperature was 100°C.

Methods. The hydroperoxides of methyl linoleate listed in Table 1 were formed by exposing methyl linoleate that was kept in a clear glass vial to air for one week at room temperature. The four hydroperoxides were separated from the starting material by thin-layer chromatography (TLC) using hexane/isopropanol (94:6, vol/vol). The mixture of hydroperoxide isomers was extracted from the silica gel with dichloromethane, concentrated and resuspended in hexane. The individual positional and geometrical hydroperoxide isomers were isolated from the mixture of hydroperoxides by HPLC and analyzed by UV spectrophotometry, NMR and negative methane chemical ionization (NCl) mass spectrometry.

The eight methyl linolenate hydroperoxide isomers listed in Table 2 were formed from methyl linolenate that

TABLE 1

Methyl Linoleate Hydroperoxide and Allylic Nitrite and Nitro Isomers

Compounds
Hydroperoxides of methyl linoleate:
Methyl 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate
Methyl 13-hydroperoxy-*trans*-9,*trans*-11-octadecadienoate
Methyl 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoate
Methyl 9-hydroperoxy-*trans*-10,*trans*-12-octadecadienoate
Allylic nitrite and nitro isomers of methyl linoleate:
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 9-nitro-*trans*-10,*cis*-12-octadecadienoate
Methyl 9-nitro-*trans*-10,*cis*-12-octadecadienoate

TABLE 2

Methyl Linolenate Hydroperoxide Isomers and Their Abbreviations

Compounds	Abbreviations
Methyl 16-hydroperoxy-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate	9*c*,12*c*,14*t*-16HP
Methyl 16-hydroperoxy-*cis*-9,*trans*-12,*trans*-14-octadecatrienoate	9*c*,12*t*,14*t*-16HP
Methyl 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate	9*c*,11*t*,15*c*-13HP
Methyl 13-hydroperoxy-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate	9*t*,11*t*,15*c*-13HP
Methyl 12-hydroperoxy-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate	9*c*,13t,15*c*-12HP
Methyl 12-hydroperoxy-*cis*-9,*trans*-13,*trans*-15-octadecatrienoate	9*c*,13*t*,15*t*-12HP
Methyl 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate	10*t*,12*c*,15*c*-9HP
Methyl 9-hydroperoxy-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate	10*t*,12*t*,15*c*-9HP

was kept in a clear glass vial and exposed to air for one week at room temperature. Mixtures of all eight of the hydroperoxide isomers were isolated by TLC from the methyl linolenate starting material. The individual hydroperoxide isomers are not resolved by adsorption phase HPLC and elute from the column as four groups of unresolved positional and geometrical isomers. Each group of unresolved hydroperoxides was isolated by HPLC and analyzed by NMR.

A sodium borohydride reduction (16) was used to reduce the four groups of unresolved geometrical and positional methyl linolenate hydroperoxide isomers eluting by HPLC as four peaks (I–IV) (Fig. 2). The eight isomeric alcohols formed by reduction are resolved by HPLC. Individual isomers were identified by their HPLC elution order, as given by Chan and Levett (16); the hydroperoxide assignments are given in Table 3.

The mixtures of allylic nitrite and nitro isomers (AN) of methyl linoleate listed in Table 1 were prepared by allowing 40 ppm of nitrogen dioxide in helium to react with purified methyl linoleate (neat, 3.4 mmole) for one hour. A bubbler apparatus similar to the one utilized in our earlier study (14) was used at a carrier gas flow rate of 60 mL/min at 25°C. The entire reaction was carried out in a glove bag under nitrogen. The nitrogen dioxide concentration in the carrier gas was determined by Saltzman analysis (18) of the thymol trap solution used in a run with no fatty acid in the apparatus. The mixture of allylic nitrite and nitro compounds was separated from the methyl linoleate starting material by TLC using hexane/isopropanol (94:6, vol/vol) that had been purged of oxygen in a glove bag under an atmosphere of nitrogen. The

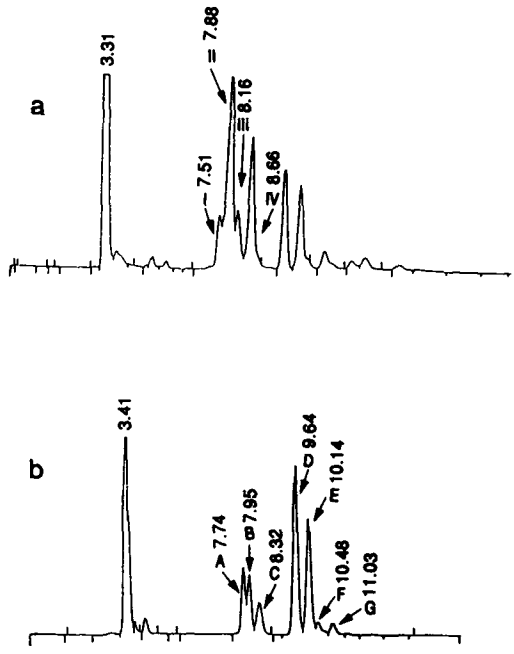


FIG. 2. High-performance liquid chromatographic chromatograms of: (a) linolenate hydroperoxide fractions that were isolated (I-IV) and (b) sodium borohydride-reduced linolenate hydroperoxides. Linolenate alcohols: A = 13-*cis,trans;* B = 12-*cis,trans;* C = 12-*trans,trans* and 13-*trans,trans;* D = 16-*cis,trans;* E = 9-*cis,trans;* F = 16-*trans,trans;* and G = 9-*trans,trans* (16).

TABLE 3

Methyl Linolenate Hydroperoxide Fraction Assignments

Fraction numbers[a]	Hydroperoxides
I	(9*c*,11*t*,15*c*-13HP)
II	(9*c*,11*t*,15*c*-13HP), (9*c*,13*t*,15*c*-12HP), (9*c*,13*t*,15*t*-12HP), (9*t*,11*t*,15*c*-13HP), and (9*c*,12*c*,14*t*-16HP)
III	(9*t*,11*t*,15*c*-13HP) and (9*c*,12*t*,14*t*-16HP)
IV	(10*t*,12*c*,15*c*-9HP) and (10*t*,12*t*,15*c*-9HP)

[a]A 25 × 0.46 cm silica column with hexane/isopropanol (99:1, vol/vol) at a flow rate of 1 mL/min was used for high-performance liquid chromatography (HPLC) analysis. The four fractions refer to the four peaks shown in the HPLC chromatogram in Figure 4b.

positional and geometrical isomers, which contained unresolved nitrite and nitro isomers, were isolated by HPLC.

The allylic nitrite and nitro isomers (AN) of methyl linolenate that were identified are listed in Table 4. These isomers were prepared by allowing purified methyl linolenate to react with 3 ppm of nitrogen dioxide in ultrapure helium for one hour. The mixture of allylic nitrite and nitro isomers was isolated from the starting material by TLC. The allylic nitrite and nitro isomers of methyl linolenate elute in HPLC as four peaks containing mixtures of unresolved positional and geometrical nitrite and nitro isomers. The four groups of unresolved isomers were analyzed by NMR and negative methane chemical ionization.

RESULTS AND DISCUSSION

Methyl linoleate allylic nitrite (nitro) and hydroperoxide isomers. The HPLC trace of the allylic nitrite (nitro) and hydroperoxide isomers of methyl linoleate are presented in Figure 3. The comparable hydroperoxide and allylic nitrite and nitro compounds elute at similar retention times.

Negative chemical ionization (NCI) mass spectrometry was used to distinguish these two types of compounds. NCI has previously been used for the analysis of various types of nitro and chlorinated compounds and organophosphates (19–24). The NCI spectra for the allylic nitrite (nitro) isomers of methyl linoleate (Table 5) display a *m/z* 46 and *m/z* 62 ion that is not detected in the NCI spectra for the hydroperoxides (Table 5). The *m/z* 46 ion is formed by dissociative electron capture and is a characteristic ion for nitro-containing compounds (21,23,24). The *m/z* 62 ion is indicative of a nitrate function (21) and is though to originate from homolysis of the O–N bond of the allylic nitrite isomers (25). The formation of the alkoxyl radical in the source of the mass spectrometer is followed by the termination of the radical with NO_2 to form the allylic nitrate. The allylic nitrate then undergoes C–O bond scission in the source of the mass spectrometer to give the *m/z* 62 ion. The *m/z* 62 ion is believed to be formed in the source rather than to originate from an allylic nitrate present in the sample, because a *m/z* 355 ion indicative of the M^- molecular ion for the allylic nitrate was not detected.

TABLE 4

Allylic Nitrite(nitro) Isomers of Methyl Linolenate and Their Abbreviations

Compounds	Abbreviations
Methyl 16-nitrito-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate Methyl 16-nitro-*cis*-9,*cis*-12,*trans*-14-octadecatrienoate	9*c*,12*c*,14*t*-16AN
Methyl 16-nitrito-*cis*-9,*trans*-12,*trans*-14-octadecatrienoate Methyl 16-nitro-*cis*-9,*trans*-12,*trans*-14-octadecatrienoate	9*c*,12*t*,14*t*-16AN
Methyl 13-nitrito-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate Methyl 13-nitro-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate	9*c*,11*t*,15*c*-13AN
Methyl 13-nitrito-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate Methyl 13-nitro-*trans*-9,*trans*-11,*cis*-15-octadecatrienoate	9*t*,11*t*,15*c*-13AN
Methyl 12-nitrito-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate Methyl 12-nitro-*cis*-9,*trans*-13,*cis*-15-octadecatrienoate	9*c*,13*t*,15*c*-12AN
Methyl 12-nitrito-*cis*-9,*trans*-13,*trans*-15-octadecatrienoate Methyl 12-nitro-*cis*-9,*trans*-13,*trans*-15-octadecatrienoate	9*c*,13*t*,15*t*-12AN
Methyl 9-nitrito-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate Methyl 9-nitro-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate	10*t*,12*c*,15*c*-9AN
Methyl 9-nitrito-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate Methyl 9-nitro-*trans*-10,*trans*-12,*cis*-15-octadecatrienoate	10*t*,12*t*,15*c*-9AN

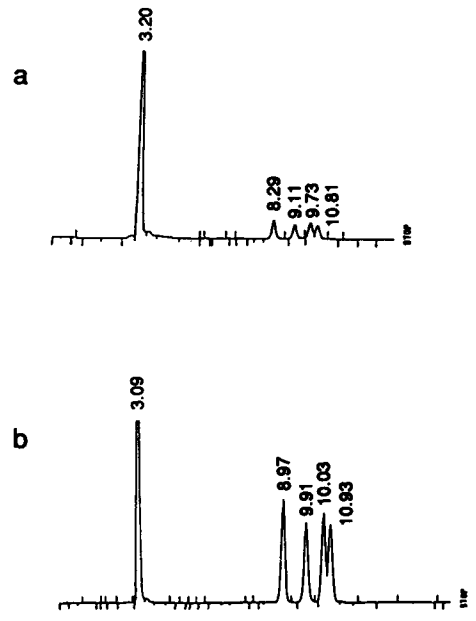


FIG. 3. High-performance liquid chromatographic chromatograms of the (a) allylic nitrite(nitro) isomers and (b) hydroperoxide isomers.

The *m/z* 339 M^- molecular ion of the allylic nitrite (nitro) isomers formed by resonance capture (23) (Table 5) reflects the ability of the compound to stabilize a negative charge (23). The *m/z* 326 molecular ion of the hydroperoxides (Table 5) was not detected. The $[M - H]^-$ ion of *m/z* 338 for the allylic nitrite (nitro) and *m/z* 325 ion for the hydroperoxides of methyl linoleate is caused by dissociative electron capture (23). The $[M + H]^-$ *m/z* 340 ion in the spectra of the allylic nitrites (nitro) is formed by H-abstraction reactions of the M^- ion (22). The Formation of $[M + H]^-$ ions also were reported by Dougherty and Dalton (22) for all chlorinated compounds that form a molecular ion. Consequently, the hydroperoxide isomers do not exhibit an M^- ion or an $[M + H]^-$ ion of *m/z* 327.

Determining the position of the hydroperoxide and nitrite functional groups. The positions of the nitrite group in the allylic nitrite isomers and of the HOO^- group in the hydroperoxide isomers were determined by the formation of an aldehyde produced from the thermal homolysis of the O-N and O-O bonds in the source of the mass spectrometer. The M^- ion of the aldehydes can be seen in the NCI spectra. Hydroperoxides have been reported to undergo thermal homolysis of the O-O bond to form an alkoxyl radical in the source of the mass spectrometer (26,27) which undergoes C-C bond cleavage to

TABLE 5

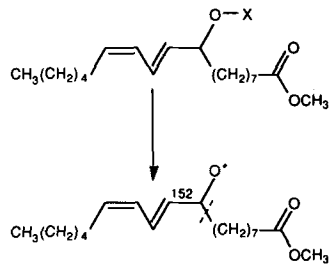
Negative Chemical Ionization Spectral Data for the Hydroperoxides and Allylic Nitrite and Nitro Compounds of Methyl Linoleate

Compounds	NO_2	NO_3	*(m/z)* 151	*(m/z)* 237	$[M - H]^-$	M^-	$[M + H]^-$
9-Hydroperoxide *(trans,trans)*	—	—	60%	—	*m/z* 325 (25%)	—	—
9-Hydroperoxide *(cis,trans)*	—	—	38%	—	*m/z* 325 (9%)	—	
9-Nitrito or (nitro) *(cis,trans)*	26%	19%	10%	—	*m/z* 338 (15%)	339 (8%)	340 (15%)
9-Nitrito or (nitro) *(trans,trans)*	78%	62%	12%	—	*m/z* 338 (21%)	339 (11%)	340 (22%)
13-Hydroperoxide *(cis,trans)*	—	—	—	53%	*m/z* 325 (9%)	—	—
13-Hydroperoxide *(trans,trans)*	—	—	—	74%	*m/z* 325 (22%	—	—
13-Nitrito or (nitro) *(cis,trans)*	72%	24%	—	25%	*m/z* 338 (20%)	339 (9%)	340 (20%)
13-Nitrito or (nitro) *(trans,trans)*	81%	40%	—	18%	*m/z* 338)12%)	339 (5%)	340 (12%)

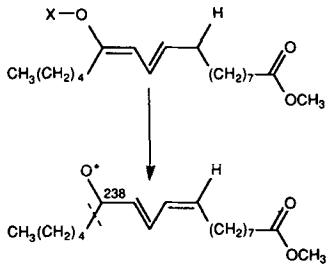
form an aldehyde (26). One of the major aldehydes reported to be formed by thermal homolysis of the 9-hydroperoxide isomers of methyl linoleate is 2,4-decadienal (26,27) (Scheme 3, where X = -OH, -NO). The major aldehyde formed by thermal homolysis of the 13-hydroperoxide of methyl linoleate is methyl 13-oxo-9,11-tridecadienoate (28,29) (Scheme 4, where X = -OH, -NO). The 9- and 13-positional allylic nitrite isomers also can form the same intermediate alkoxyl radicals that can undergo C-C bond scission to form 2,4-decadienal and methyl 13-oxo-9,11-tridecadienoate (Schemes 3 and 4).

In the NCI spectra for the 9- and 13-hydroperoxides (Table 5), a $[M - H]^-$ ion of *m/z* 151 for 2,4-decadienal is detected for the 9-hydroperoxides. Correspondingly, the $[M - H]^-$ ion of *m/z* 237 for methyl 13-oxo-9,11-tridecadienoate is seen in the NCI spectra for the 13-hydroperoxides (Table 5). The $[M - H]^-$ *m/z* 151 ion of 2,4-decadienal also is detected in the NCI spectra for the 9-allylic nitrite(nitro) isomers (Table 5). Similarly, the $[M - H]^-$ *m/z* 237 ion for methyl 13-oxo-9,11-tridecadienoate is detected in the NCI spectra for the 13-allylic nitrite(nitro) isomers. The detection of the *m/z* 151 ion and the *m/z* 237 ion in the NCI spectra of the 9- and 13-allylic nitrite(nitro) compounds, respectively, confirms the position of the functional group.

The presence of the nitro functional group. The coelution of the allylic nitro and nitrite isomers was determined by hydrolyzing the 13-*cis,trans*-allylic nitrite(nitro) compound; alkyl nitrites are known to hydrolyze to alcohols (30). The hydrolysis products of the 13-*cis,trans*-allylic nitrite(nitro) isomers display the *m/z* 46 and *m/z* 62 ions indicative of the nitro and nitrate groups by NCI. The $[M - H]^-$ *m/z* 338 and $[M + H]^-$ *m/z* 340 ions also are present. If the sample consisted of only the allylic nitrite isomer, the *m/z* 46, *m/z* 62, *m/z* 338 and *m/z* 340 ions would not have been detected in the hydrolyzed sample since alcohols do not give these ions.



SCHEME 3



SCHEME 4

IR spectral data. Table 6 displays IR data for isopentyl nitrite, nitrocyclohexane, a mixture of isopentyl nitrite and nitrocyclohexane, and our allylic nitrite(nitro) isomers. Stretching frequencies at 1603 cm^{-1} and 1587 cm^{-1} for the allylic nitrite(nitro) isomers may be caused by the presence of an oxime (31) formed by the decomposition of the allylic nitrite isomers. The stretching frequency for the nitro functional group is 1377 cm^{-1}. The stretching frequencies we observed diverge from the normal values, perhaps because of the electron-withdrawing effects of the double bonds (32).

Maximum UV absorptions. The UV maximum absorption wavelength of allylic nitrite(nitro) isomers isolated by HPLC using hexane/isopropanol (99:1, vol/vol) is 236 ± 2 nm for the *cis,trans* isomers and 234 ± 2 nm for the *trans,trans* isomers. The methyl linoleate hydroperoxides exhibit a UV maximum at 234 ± 2 nm for the *cis,trans* and 232 ± 2 nm for the *trans,trans* in hexane/isopropanol (99:1, vol/vol); these absorptions indicate that both isomers have conjugated double bonds (33).

Proton NMR data of the allylic nitrite(nitro) and hydroperoxide isomers. The proton NMR data for the 13-*cis,trans* and 13-*trans,trans* allylic nitrite(nitro) isomers are listed in Table 7. The 13-*cis,trans* and 13-*trans,trans* hydroperoxides reported by Gardner and Plattner (28) have similar chemical shifts and splitting patterns. The proton NMR data of the 9- and 13-allylic nitrite(nitro) isomers have the same chemical shifts and splitting patterns (Table 8). The 9- and 13-hydroperoxides of methyl linoleate also are reported to have identical proton NMR spectra (28). These similarities between the proton NMR data of the allylic nitrites and hydroperoxide isomers were used to confirm the structures assigned to the allylic nitrite(nitro) isomers.

NCI of the allylic nitrite(nitro) isomers of methyl linolenate. The allylic nitrite(nitro) isomers and hydroperoxides of methyl linolenate elute at similar retention times (Fig. 4), as do the allylic nitrite(nitro) isomers and hydroperoxides of methyl linoleate (Fig. 3). NCI mass spectrometry was used to identify the allylic nitrite(nitro) isomers of methyl linolenate that elute by HPLC as four groups (labeled "fraction I-IV") of mixtures of positional and geometrical isomers.

The NCI spectral data for the mixture of allylic nitrite (nitro) isomers in fraction I are shown in Table 9. The $[M + H]^-$ ion of *m/z* 338 and M^- ion of *m/z* 337 detected in this fraction confirm the molecular weight of the allylic nitrite(nitro) isomer. The *m/z* 46 ion, indicative of a nitro group, and the *m/z* 62 ion, indicative of a nitrate, also are

TABLE 6

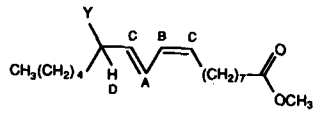
Infrared Stretching Frequencies of Nitro and Nitrite Compounds[a]

Compounds	IR frequencies (cm^{-1})
Allylic nitrite(nitro) isomers	1603, 1587, 1377
Isopentyl nitrite	1640, 1553
Nitrocylohexane	1545, 1379
Isopentyl nitrite and nitrocyclohexane	1653, 1635, 1545, 1377

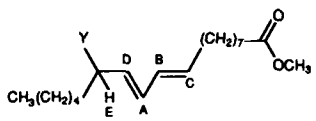
[a]The infrared (IR) spectra of the neat samples were taken with an IBM IR/45 spectrometer.

TABLE 7

Nuclear Magnetic Resonance Data of the 13-*cis,trans*- and 13-*trans,trans*-Allylic Nitrite(nitro) Compounds of Methyl Linoleate[a]



STRUCTURE 1



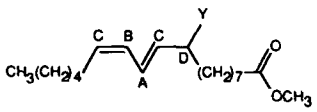
STRUCTURE 2

13-*c,t*-Allylic nitrite(nitro)	13-*t,t*-Allylic nitrite(nitro)
A = 6.58 ppm (*dd*, *J* = 15,10.5)	A = 6.29 ppm (*dd*, *J* = 15.5,10.5)
B = 6.02 ppm (*t*, *J* = 12.5,10.5)	B = 6.05 ppm (*t*, *J* = 15,12.5)
C = 5.5 ppm (*m*, *J* = 9)	C = 5.77 ppm (*dd*, *J* = 15.5,6.5)
D = 4.4 ppm (*m*, *J* = 7.5)	D = 5.47 ppm (*dd*, *J* = 15,9)
	E = 4.34 ppm (*m*, *J* = 7.5)

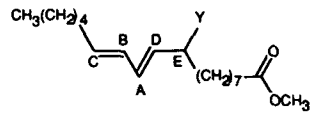
[a]*dd* = doublet doubled, *t* = triplet, *m* = multiplet; the chemical shift (mult,*J*,Hz) is given. In Structures 1 and 2, Y = NO_2, ONO.

TABLE 8

The Nuclear Magnetic Resonance Data of the 9-*cis,trans*-Allylic Nitrite(nitro) and 9-*trans,trans*-Allylic Nitrite(nitro)[a]



STRUCTURE 3



STRUCTURE 4

9-*c,t*-Allylic nitrite(nitro)	9-*t,t*-Allylic nitrite(nitro)
A = 6.58 ppm, (*dd*, *J* = 15,10.5)	A = 6.29 ppm (*dd*, *J* = 15.5,10.5)
B = 6.02 ppm, (*t*, *J* = 12.5,10.5	B = 6.05 ppm (*t*, *J* = 15,12.5)
C = 5.5 ppm, (*m*, *J* = 9)	C = 5.77 ppm (*dd*, *J* = 15.5,6.5)
D = 4.4 ppm, (*m*, *J* = 7.5)	D = 5.47 ppm (*dd*, *J* = 15,9)
	E = 4.34 ppm (*m*, *J* = 7.5)

[a]*dd* = doublet doubled, *t* = triplet, *m* = multiplet; the chemical shifts (mult/*J*, Hz) are given. In Structures 3 and 4, Y = NO_2, ONO.

TABLE 9

Negative Chemical Ionization Mass Spectral Data for the Allylic Nitrite and Nitro Compounds of Methyl Linolenate

Fractions[a]	NO_2	NO_3	(*m/z*) 109	(*m/z*) 149	(*m/z*) 237	(*m/z*) 277	M^-	$[M + H]^-$
I	72%	2%	—	—	70%	—	6%	18%
II	17%	2%	2%	—	—	37%	<1%	<1%
III	100%	2%	—	—	5%	12%	5%	10%
IV	98%	12%	—	76%	—	—	10%	20%

[a]The four fractions refer to the four peaks in the chromatogram in Figure 4a.

detected. The $[M - H]^-$ ion of methyl 13-oxotridecadienoate, *m/z* 237, is formed by O–N homolysis and subsequent carbon-carbon bond scission of the 13-allylic nitrite isomer (Scheme 5).

The NCI spectral data for the mixture of allylic nitrite (nitro) isomers in fraction II are shown in Table 9. The $[M + H]^-$ ion at *m/z* 338, the parent ion at *m/z* 337, the NO_2^- ion at *m/z* 46 and the NO_3^- ion at *m/z* 62 are observed. The *m/z* 109 ion is the $[M - H]^-$ ion of 2,4-heptadienal formed by thermal homolysis of the 12-allylic nitrite isomer(s) (Scheme 6). The *m/z* 277 ion is the $[M - H]^-$ ion of methyl 16-oxo-9,12,14-hexadecatrienoate isomer(s) formed by thermal homolysis of the 16-allylic nitrite isomer (Scheme 7).

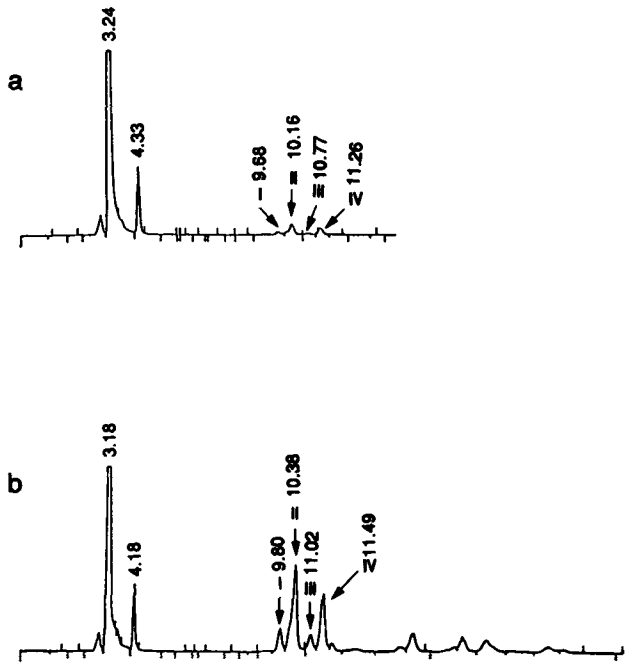
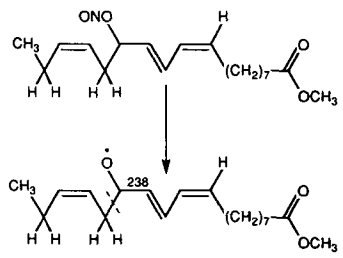
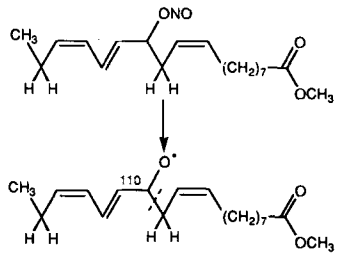


FIG. 4. High-performance liquid chromatographic chromatograms of methyl linolenate (a) allylic nitrite(nitro) isomers and (b) hydroperoxide isomers.



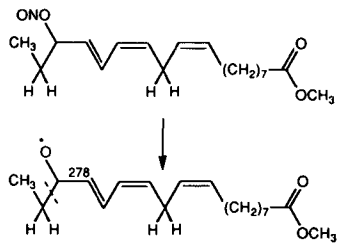
SCHEME 5



SCHEME 6

The NCI spectral data for the mixture of allylic nitrite(nitro) isomers in fraction III are shown in Table 9. The $[M + H]^-$ ion of *m/z* 338, M^- ion of *m/z* 337, NO_2^- ion *m/z* 46, and NO_3^- ion of *m/z* 62 are all observed. The *m/z* 237 ion for the 13-allylic nitrite isomer and the *m/z* 277 ion for the 16-allylic nitrite isomer were both detected.

The NCI spectral data of the mixture of allylic nitrite(nitro) isomers in fraction IV are shown in Table 9.



SCHEME 7

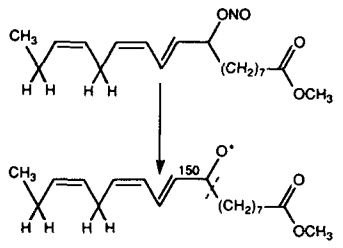
The $[M + H]^-$ ion of *m/z* 338, M^- ion of *m/z* 337, NO_2^- ion of *m/z* 46 and the NO_3^- ion of *m/z* 62 are detected. The *m/z* 149 ion is the $[M - H]^-$ ion of 2,4,7-decatrienal formed by thermal homolysis of the 9-allylic nitrite isomer (Scheme 8).

Proton NMR analysis of the hydroperoxide and allylic nitrite(nitro) fractions of methyl linolenate. The proton NMR chemical shifts and splitting patterns of the olefinic region of the hydroperoxide fractions of methyl linolenate are similar to those of the allylic nitrite(nitro) isomers (Table 10). The proton NMR of the methyl linoleate hydroperoxides and allylic nitrite(nitro) isomers also are very similar.

Assignments of the allylic nitrite(nitro) isomer fractions of methyl linolenate. The assignments of the allylic nitrite (nitro) isomers of methyl linolenate obtained from the NCI and NMR data presented above are listed in Table 11. Since the isomers of the methyl linoleate hydroperoxides and allylic nitrite isomers of methyl linoleate elute at similar retention times, the assigned *cis,trans* and *trans,trans* geometries of the allylic nitrite (nitro) isomers of methyl linolenate were based on the similar elution time profile of the hydroperoxide isomers.

We have shown that nitrogen dioxide reacts with methyl linoleate and linolenate in the absence of oxygen to give allylic nitro and nitrite isomers (34). Our data demonstrate that these isomers can be identified by NCI mass spectrometry. We have previously rationalized these products by a mechanism involving hydrogen atom abstraction by nitrogen dioxide (14,15).

The allylic nitrite(nitro) compounds and hydroperoxides elute at similar retention times by adsorption HPLC. The UV maximum absorption wavelengths and the proton NMR spectra of the allylic nitrite(nitro) and hydroperoxide compounds also are similar. These similarities were used to confirm the structure of the nitrite(nitro) compounds.



SCHEME 8

TABLE 10

Proton Nuclear Magnetic Resonance Data of the Hydroperoxide and Allylic Nitrite(nitro) Fractions of Methyl Linolenate[a]

Fraction II[b]		Fraction IV[c]	
ROOH	RNO_2/RONO	ROOH	RNO_2/RONO
6.6 ppm *(m)*	6.6 ppm *(m)*	6.6 ppm *(m)*	6.6 ppm *(m)*
6.05 ppm *(t)*	6.05 ppm *(t)*	6.05 ppm *(t)*	6.05 ppm *(t)*
5.3-5.7 ppm *(br,m)*	5.3-5.65 ppm *(br,m)*	5.3-5.65 ppm *(br,m)*	5.3-5.65 ppm *(br,m)*
4.35 ppm *(br,m)*	4.35 ppm *(m)*	4.4 ppm *(m)*	4.38 *(m)*

[a] *br* = broad, *m* = multiplet and *t* = triplet. Since the fractions are a mixture of isomers, the coupling constants are not shown.

[b] Fraction II for the hydroperoxides and allylic nitrite(nitro) isomers was isolated using a silica column with hexane/isopropanol (99:1, vol/vol) at a flow rate of 1 mL/min. The retention time for the hydroperoxide in Figure 4b was 10.38 min and for the allylic nitrite(nitro) in Figure 4a was 10.16 min.

[c] The retention time of fraction IV is 11.49 min for the hydroperoxide and 11.26 min for the allylic nitrite(nitro) isomer.

TABLE 11

Allylic Nitrite(nitro) Isomers of Methyl Linolenate Contained in Isolated Fractions[a]

Fractions	Allylic nitrite(nitro) isomers
I	(9*c*,11*t*,15*c*-13AN)
II	(9*c*,13*t*,15*c*-12AN), (9*c*,13*t*,15*t*-12AN), and (9*c*,12*c*,14*t*-16AN)
III	(9*t*,11*t*,15*c*-13AN) and (9*c*,12*t*,14*t*-16AN)
IV	(10*t*,12*c*,15*c*-9AN) and (10*t*,12*t*,15*c*-9AN)

[a] A 25 × 0.46 cm silica column with hexane/isopropanol (99:1, vol/vol) at a flow rate of 1 mL/min was used for high-performance liquid chromatography (HPLC) analysis. The four peaks refer to the HPLC trace in Figure 4a.

Negative methane chemical ionization (NCI) was used to identify the presence of a nitrite and nitro group, to confirm the molecular weight and to locate the position of the functional group. The NCI spectra for the allylic nitrite(nitro) isomers of methyl linoleate (Table 5) and methyl linolenate (Table 9) display a *m/z* 46 and *m/z* 62 ion indicative of a nitro and nitrate functional group (20,22,23) and *m/z* 339 and *m/z* 337 molecular ions. These ions were not seen in the NCl spectra for the hydroperoxides of methyl linoleate (Table 5). The position of the functional group was determined by the aldehyde formed by thermal homolysis of the O-N bond of the nitrite group.

ACKNOWLEDGMENTS

We would like to thank Dr. S. Barker, Dr. R. Laine and T. Meyer for the use of their mass spectrometer and their assistance in analyzing the compounds. This work was supported in part by a MERIT Award from the National Institutes of Health, HL-16029.

REFERENCES

1. Dawson, S.V., and Schenker, M.B. (1979) *Am. Rev. Respir. Dis. 120,* 281-292.
2. Guidotti, T.L. (1978) *Environ. Res. 15,* 443-472.
3. Richter, A., and Kuratis, K. (1981) *Arch. Environ. Health 36,* 36-39.
4. Richter, A., and Richter, V. (1983) *Arch. Environ. Health 38,* 69-75.
5. Richter, A., and Kuratis, K. (1983) *Environ. Health Perspect. 52,* 165-168.
6. Richter, A. (1988) *J. Toxicol. Environ. Health 25,* 383-390.
7. Patel, J.M., and Block, E.R. (1986) *Am. Rev. Respir. Dis. 134,* 1196-1202.
8. Stephens, R.J., Freeman, G., and Evans, M. (1972) *Arch. Environ. Health 24,* 160-179.
9. Freeman, G., Juhos, L.T., Furiosi, N.J., Mussenden, R., Stephens, R.J., and Evans, M.J. (1974) *Arch. Environ. Health 29,* 203-210.
10. Thomas, H.V., Mueller, P.K., and Lyman, R.L. (1968) *Science 159,* 532-534.
11. Postlethwait, E., and Bidani, A. (1989) *Toxicol. Appl. Pharmacol. 98,* 303-312.
12. Kikugawa, K., and Kogi, K. (1989) *Chem. Pharm. Bull. 35,* 344-349.
13. Mead, J.R. (1976) in *Free Radicals in Biology* (Pryor, W.A., ed.) Vol. 1, pp. 51-68, Academic Press, New York.
14. Pryor, W.A., Lightsey, J.W., and Church, D.F. (1982) *J. Am. Chem. Soc. 104,* 6685-6692.
15. Pryor, W.A., and Lightsey, J.W. (1981) *Science 214,* 435-437.
16. Chan, H.W.S., and Levett, G. (1977) *Lipids 12,* 837-840.
17. Porter, N.A., and Wujek, D.G. (1984) *J. Am. Chem. Soc. 106,* 2626-2629.
18. Saltzman, B.E. (1954) *Anal. Chem. 26,* 1949-1955.
19. Tannenbaum, H.P., Roberts, J.D., and Dougherty, R.C. (1975) *Anal. Chem. 47,* 49-54.
20. Rankin, P.C. (1971) *J. Assoc. Off. Anal. Chem. 54,* 1340-1348.
21. Bouma, W.J., and Jennings, K.R. (1981) *Org. Mass Spectrom. 16,* 331-335.
22. Dougherty, R.C., and Dalton, J. (1972) *Org. Mass Spectrom. 6,* 1171-1181.
23. Dillard, J.G. (1973) *Chem. Rev. 73,* 589-643.
24. Yinon, J., and Boettger, H.G. (1972) *Anal. Chem. 44,* 2235-2237.
25. Gray, P. (1955) *Trans. Faraday Soc. 51,* 1367-1374.
26. Frankel, E.N., Neff, W.E., and Selke, E. (1981) *Lipids 16,* 279-285.

27. Chan, H.W.S., Prescott, A.A., and Swoboda, P.A.A. (1976) *J. Am. Oil Chem. Soc. 53,* 572–576.
28. Gardner, H.W., and Plattner, R.D. (1984) *Lipids 19,* 294–299.
29. Plattner, R.D., and Gardner, H.W. (1985) *Lipids 20,* 126–131.
30. March, J. (1985) *Advanced Organic Chemistry,* 3rd edn., p. 739, John Wiley & Sons Inc., New York.
31. Silverstein, R.M., Bassler, G.C., and Morril, T.C. (1981) *Spectrometric Identification of Organic Compounds,* 4th edn., p. 130, John Wiley & Sons Inc., New York.
32. Pouchert, C.J. (1981) *The Aldrich Library of Infrared Spectra,* 3rd edn., p. 1325, Aldrich Chemical Co., Milwaukee, Wisconsin.
33. Chan, H.W.S., and Levett, G. (1976) *Lipids 12,* 99–104.
34. Gallon, A.A. (1990) The Mechanism of Low Levels of Nitrogen Dioxide Reactions with Polyunsaturated Fatty Acids Esters, Dissertation, Louisiana State University, Baton Rouge, pp. 29–151.

[Received November 15, 1991, and in revised form October 22, 1992; Revision accepted November 30, 1992]