Acid-Promoted Reactions of Ethyl Linoleate with Nitrite Ions: Formation and Structural Characterization of Isomeric Nitroalkene, Nitrohydroxy, and Novel 3-Nitro-1,5-hexadiene and 1,5-Dinitro-1,3-pentadiene Products

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The reaction of ethyl linoleate (**1**) with NO_2^- in different air-equilibrated acidic media resulted in the formation of complex patterns of products, some of which could be isolated by repeated TLC fractionation and were formulated as the nitroalkenes **2**−**5**, the novel (1*E*, 5*Z*)-3-nitro-1,5-hexadienes **6**/**7**, the novel (*E*,*E*)-1,5-dinitro-1,3-pentadiene derivatives **8** and **9**, and the nitro alcohols **10**/**11** and **12**/**13** by extensive GC−MS and 2D NMR analysis, as aided by 1D Hartmann−Hahn proton mapping experiments. Similar reaction of methyl oleate gave mainly nitroalkene (**14/15**) and allylic nitro derivatives (**16/17**). Formation of **2**−**13** may be envisaged in terms of HNO_2-mediated nitration pathways in which regioisomeric β-nitroalkyl radical intermediates derived from attack of NO_2 to the 1,4-pentadiene moiety of **1** evolve through competitive H-atom abstraction and free radical combination routes.

Introduction

Nitration reactions targeted to polyunsaturated fatty acids and related membrane lipid components attract continuing interest as putative mechanisms of cytotoxicity and cell damage evoked by nitrogen oxides from both metabolic and environmental sources. Studies aimed at modeling acute and chronic exposures of pulmonary lipids to NO_2 in severely polluted urban atmosphere[1−3] provided evidence that at low ppm levels NO_2 reacts with unsaturated fatty acids predominantly by H-atom abstraction leading to allylic nitration products and/or hydroperoxides,[4,5] whereas at relatively high concentrations it may add reversibly to the double bond to give β-nitroalkyl radicals.

More recent work showed that nitric oxide[6,7] (nitrogen monoxide, NO), a powerful biological effector, and oxidation products thereof, viz. peroxynitrite, NO_2, HNO_2 and NO_2^+, interact with linoleic acid and lipid peroxides to give complex mixtures of products, including nitroepoxides and other nitrogen containing derivatives of oxidized lipids.[8−10] At high concentrations and in the presence of oxygen, NO reacts with polyunsaturated fatty acids and esters to afford mixtures of nitration products, including isomeric nitroalkene and nitronitrate derivatives.[11,12] Yet, the biological and toxicological properties of nitrated fatty acid derivatives have remained largely unexplored, also because of the lack of convenient synthetic approaches: in fact, not a single nitration product of polyunsaturated fatty acids has so far been isolated in pure form and structurally characterized.

In searching for a practical access to nitrated lipid derivatives, we found that ethyl linoleate (**1**) reacts smoothly with NO_2^- in acidic media, viz. under conditions favoring formation of HNO_2, to afford complex, yet relatively well-defined patterns of nitration products, some of which were amenable to chromatographic isolation. A review of the literature revealed a virtual lack of systematic studies of the reaction of HNO_2 with polyunsaturated fatty acids or related diene compounds, the main relevant record dealing with the inhibitory properties of methyl linoleate on HNO_2^-induced nitrosamine formation.[13] Additional incentives for studies of this reaction derived from its possible relevance to the toxic effects caused by high levels of NO_2^- from preserved/pickled foods or polluted drinking water in the acidic gastric compartment (pH 2.5−4.5).[9,14−16] Similar NO_2^--mediated processes may be implicated in the mechanisms of tissue injury in sepsis, skin inflammation, cerebral

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(1) Pryor, W. A.; Lightsey, J. W. *Science* **1981**, *214*, 435−437.
(2) Pryor, W. A.; Lightsey, J. W.; Church, D. F. *J. Am. Chem. Soc.* **1982**, *104*, 6685−6692.
(3) Giamalva, D. H.; Kenion, G. B.; Church, D. F.; Pryor, W. A. *J. Am. Chem. Soc.* **1987**, *109*, 7059−7063.
(4) Gallon, A. A.; Pryor, W. A. *Lipids* **1993**, *28*, 125−133.
(5) Gallon, A. A.; Pryor, W. A.*Lipids* **1994**, *29*, 171−176.
(6) Pfeiffer, S.; Mayer, B.; Hemmens, B. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1714−1731.
(7) Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev.* **1993**, *22*, 233−241.
(8) O'Donnell., V.B.; Eiserich, J. P.; Chumley, P. H.; Jablonsky, M. J.; Krishna, N. R.; Kirk, M.; Barnes, S.; Darley-Usmar, V. M.; Freeman, B. A. *Chem. Res. Toxicol.* **1999**, *12*, 83−92.
(9) O'Donnell., V.B.; Eiserich, J. P.; Bloodsworth, A.; Chumley, P. H.; Kirk, M.; Barnes, S.; Darley-Usmar, V. M.; Freeman, B. A. *Methods Enzymol.* **1999**, *301*, 454−470.
(10) Rubbo, H.; Radi, R.; Trujillo, M.; Telleri, R.; Kalyanaraman, B.; Barnes, S.; Kirk, M.; Freeman, B. A. *J. Biol. Chem.* **1994**, *269*, 26066−26075.
(11) d'Ischia, M.; Rega, N.; Barone, V. *Tetrahedron* **1999**, *55*, 9297−9308.
(12) d'Ischia, M. *Tetrahedron Lett.* **1996**, *37*, 5773−5774.
(13) Kikugawa, K.; Kato, T.; Konoe, Y.; Sawamura, A. *Food Chem. Toxicol.* **1985**, 23, 339−342.

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Table 1. Selected NMR Data of Compound 2

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2−	28.69	2.21 (m)	7.08, 1.50	7.08
−*C*H=CNO_2−	137.15	7.08 (t, 8.0)	2.21	3.33, 2.21, 1.50
−CH=*C*NO_2−	151.45			7.08, 3.33, 2.21
=CNO_2CH_2CH=	25.65	3.33 (d, 6.8)	5.26	7.08, 5.48, 5.26
−CH_2*C*H=CH−	124.04	5.26 (m)	5.48, 3.33	3.33, 2.13
−CH_2CH=*C*H−	133.79	5.48 (m)	5.26, 2.13	3.33, 2.13, 1.35
−CH_2-R^2	28.20	2.13 (m)	5.48, 1.35	5.48, 5.26

ischemia,and other pathological conditions associated with up-regulated nitric oxide synthase activity, abnormal accumulation of NO_2^-, and local drops of pH.[9, 16, 17]

The purpose of this investigation was to provide a detailed structural characterization of the main products formed by reaction of **1** with NO_2^- under acidic conditions, and to gain a short-cut access to nitrated derivatives to be tested preliminarily for cytotoxicity.

Results and Discussion

Acid-Promoted Reaction of 1 with NO_2^-. Reaction of **1** with NO_2^- in different acidic, air-equilibrated media afforded qualitatively similar patterns of products (TLC), most of which positive to the Griess reagent for nitrosating species.[18] Of the various media investigated, a 1% sulfuric acid/cyclohexane biphasic system proved the most convenient for gram scale reactions with regard to the extent of substrate conversion and ease of work up, and was preferably chosen for investigative purposes. Typically, **1** (2.0 g, 6.5 mmol) was allowed to react with NO_2^- (32.5 mmol) under vigorous stirring and progress of the reaction was monitored by TLC. Flash chromatography of the resulting cyclohexane−extractable material eventually afforded four main fractions. Fraction I (30% w/w) contained virtually pure unreacted **1** whereas fractions II−IV consisted of intimate mixtures of products and were subjected to repeated TLC fractionation. More polar fractions were also collected which, however, were made up of chromatographically ill-defined products and their identity was not investigated.

Fraction II. Repeated TLC on silica gel and $AgNO_3$-impregnated silica gel plates afforded eventually a fraction consisting of a main product (ca. 1%, R_f = 0.47, cyclohexane/ethyl acetate 95:5) which was identified as ethyl (9*E*,12*Z*)-10-nitrooctadeca-9,12-dienoate (**2**). The product (FT-IR 1520, 1337 cm^{-1}) exhibited in the ^1H NMR spectrum as most salient features a triplet at δ 7.08, typical of protons on *E*-nitroalkene functionalities, and a 2H doublet at δ 3.33, diagnostic of bis-allylic methylene protons experiencing the de-shielding effect of an adjacent nitro group (Table 1).[11,12] The position of the nitro group was deduced on the basis of Hartmann−Hahn[19] and COSY proton mapping experiments, which identified

Table 2. Selected NMR Data of Compound 3

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^2-CH_2−	28.89	2.21 (m)	7.12, 1.50	7.12, 1.50
−*C*H=CNO_2−	137.73	7.12 (t, 8.0)	2.21	3.27, 2.21, 1.50
−CH=*C*NO_2−	151.04			7.12, 3.27, 2.21
=CNO_2CH_2CH=	29.85	3.27 (d, 6.0)	5.37, 2.22, 1.98	7.12, 5.48, 5.27.
−CH_2*C*H=CH−	124.47	5.37 (m)	5.48, 3.27	5.48, 3.27, 1.98
−CH_2CH=*C*H−	134.28	5.48 (m)	5.37, 3.27, 1.98	3.27, 1.98
−CH_2-R^1	33.09	1.98 (m)	5.48, 3.27, 1.35	5.48, 5.37

Table 3. Selected NMR Data of Compounds 4 and 5[a]

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2−	27.1	2.58 (t, 7.6)	1.50	7.03, 7.02, 1.50
−CNO_2=CH−	153.7			2.95, 2.58
−CNO_2=*C*H−	135.3	7.03[b] (t, 8.0)	2.90	2.95, 2.90, 2.58
		7.02[c] (t, 8.0)	2.95	
=CH*C*H_2CH=	26.9	2.95[c] (t, 7.6)	5.35	
		2.90[b] (t,7.6)	5.36	
−CH_2*C*H=CH−	124.1	5.36[b] (m)	5.53, 2.90	2.95, 2.90, 2.05
		5.35[c] (m)	5.52, 2.95	
−CH_2CH=*C*H−	134.3	5.53[b] (m)	5.36, 2.05	2.05
		5.52[c] (m)	5.35, 2.05	
−CH_2-R^2	28.2	2.05	5.53, 5.52	5.53,5.52, 5.36, 5.35

[a] R^1, R_2 = $-(CH_2)_3CH_3$ or $-(CH_2)_6CO_2Et$. [b] Minor isomer. [c] Main isomer.

two distinct sequences of coupled resonances, the first connecting the triplet at δ 7.08 to the C-2 triplet at δ 2.28, and the second connecting the C-18 multiplet at δ 0.92 to the C-11 doublet at δ 3.33 through the olefinic protons at δ 5.48 and 5.26.

Using a similar chromatographic protocol it was possible to isolate another fraction which contained a regioisomer of **2**, ethyl (9*Z*,12*E*)-12-nitrooctadeca-9,12-dienoate (**3**, R_f = 0.53, FT-IR 1520, 1337 cm^{-1}). The ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **2** (Table 2), and the position of the nitro group was confirmed by 1D Hartmann−Hahn experiments.

Close inspection of crude fraction II revealed additional identifiable products which however resisted all efforts at a complete separation and were therefore characterized as a mixture. Two of these products (R_f = 0.57−0.50) exhibited spectral data consistent with the 9- and 13-substituted positional isomers of **2** and **3**, and were formulated accordingly as **4** and **5**. In accord with the proposed structures, the ^1H NMR spectra exhibited, besides the protons on the *E*-nitroalkene moieties, deshielded triplets at δ 2.58, due to allylic methylene groups shifted downfield by the vicinal nitro group, whereas the doubly allylic protons appeared as triplets at δ 2.90 and 2.95, experiencing the modest influence of the remote nitro group. Complete assignment of the proton and carbon resonances belonging to each isomer was however precluded by the difficulties derived from dealing with complex mixtures of products (Table 3).

A rapid glance at the energy-minimized stereostructures of **2**−**5** (MM+ calculations) revealed for the nitroalkene moieties dihedral CCNO angles in the range of 21°−23°, which indicated that the nitro group was twisted out of the plane of the double bond, the effect being comparable for the various isomers.

(14) van der Vliet, A.; Eiserich, J. P.; Halliwell, B.; Cross, C. E. *J. Biol. Chem*. **1997**, *272*, 7617−7625.
(15) Klebanoff, S. J. *Free Rad. Biol. Med*. **1993**, *14*, 351−360.
(16) Yoshida, Y.; Hirose, M.; Takaba, K.; Kimura, J.; Ito, N. *Int. J. Cancer* **1994**, *56*, 124−128
(17) Matsumoto, T.; Obrenovitch, T. P.; Parkinson N. A.; Symon, L. *Stroke* **1990**, *21*, 1192−1998.
(18) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem*. **1982,** *126*, 131−138.
(19) Kessler, H.; Oschkinat, H.; Griesinger, C. Bermel, W. *J. Magn. Reson*. **1986**, *70*, 106−133.

Table 4. Selected NMR Data of Compounds 6 and 7[a]

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2-	28.19	2.12 (m)	5.83, 5.63	5.63
$-CH{=}CHCHNO_2-$	139.96	5.83 (m)	5.63, 2.12	5.63, 4.85, 2.12
$-CH{=}CHCHNO_2-$	124.95	5.63 (m)	5.83, 4.85	4.85, 2.84, 2.54
$-CHNO_2CH_2CH{=}$	90.41	4.85 (m)	5.63, 2.84, 2.54	5.83, 2.84, 2.54
$-CH_2CH{=}CH-$	32.62	2.54 (m), 2.84 (m)	5.26, 4.85	5.83, 5.63, 4.85
$-CH_2CH{=}CH-$	122.53	5.26 (m)	2.84, 2.54, 2.00	4.85, 2.84, 2.54, 198
$-CH_2CH{=}CH-$	135.64	5.56 (m)	5.26, 1.98	2.84, 2.54, 1.98
$-CH_2$-R^2	28.13	1.98 (m)	5.56, 5.26	5.56, 5.26

[a] R^1, $R_2 = -(CH_2)_2CH_3$ or $-(CH_2)_6CO_2Et$.

Table 5. Selected NMR Data of Compound 8

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2-	33.15[a]	1.78 (m)	5.41	6.18
$-CHNO_2CH{=}$	84.10	5.41 (m)	6.18, 1.78	6.48, 6.18
$-CHNO_2CH{=}$	140.55	6.18 (dd, 15.2, 7.2)	6.48, 5.41	7.48
$-CHNO_2CH{=}CH-$	127.52	6.48 (dd, 15.2, 11.6)	7.48, 6.18	5.41
$=CHCH{=}CNO_2-$	131.01	7.48 (d, 11.6)	6.48	6.18
$=CHCH{=}CNO_2-$	153.70			7.48
$-CH_2$-R^2	27.51	2.69 (t, 6.2)	1.61	7.48

[a] Poorly discernible in the $^1H-^{13}C$ HETCOR spectrum, identified in the HMBC spectrum.

In addition to **2**−**5**, fraction II contained a distinct Griess-positive species ($R_f = 0.50$) which could be easily identified in the ^1H NMR spectrum by a characteristic pattern of resonances comprising two multiplets for vicinal protons on a trans double bond at δ 5.83 and 5.63, two multiplets at δ 5.56 and 5.26, partially obscured by overlapping resonances, and a multiplet at δ 4.85 coupled to a pair of diastereotopic geminal protons at δ 2.84 and 2.54 (Table 4). Close analysis of $^1H-^1H$ COSY and $^1H-^{13}C$ HETCOR spectra indicated a (1*E*,5*Z*)-3-nitro-1,5-hexadiene moiety, as in structures **6** or **7**. Unfortunately, all attempts to distinguish the resonances pertaining to each of the possible regioisomers proved fruitless.

Fraction III. Fraction III contained comparable amounts of two UV-absorbing products ($R_f = 0.40$ and 0.36) that could eventually be obtained with at best 50% purity each, the remainder of the fractions being made up of unidentifiable components. The products displayed virtually identical ^1H NMR spectra featuring a characteristic set of resonances around δ 7.5 (d), 6.5 (dd), 6.2 (dd), and 5.4 (m). The FT-IR spectra exhibited two distinct sets of bands denoting nitro groups on sp^3 and sp^2 carbons. On these grounds, based also on Hartmann−Hahn experiments, the products were readily formulated as **8** and **9** containing the novel (1*E*,3*E*)-1,5-dinitro-1,3-pentadiene moiety (Tables 5 and 6). Brief investigation of the conformational properties (MM+) of **8** and **9** revealed a much more accentuated out-of-plane twisting of the nitro group in the nitroalkene moieties (dihedral CCNO angles in the range of 58°−60°) compared to compounds **2**−**5**. This presumably descends from the relative conformational rigidity of the conjugated nitrodiene moiety in **8** and **9**, causing substantial loss of flexibility of the octadecyl chain.

Table 6. Selected NMR Data of Compound 9

carbon	δ_C	δ_H(mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2-	33.11[a]	1.75 (m)	5.40, 1.35	5.40
$-CHNO_2CH{=}$	84.12	5.40 (m)	6.47, 6.17, 1.75	6.47, 6.17
$-CHNO_2CH{=}$	140.29	6.17 (dd, 15.2, 7.2)	6.47, 5.40	7.47, 5.40
$-CHNO_2CH{=}CH-$	127.64	6.47 (dd, 15.2, 11.2)	7.47, 6.17, 5.40	5.40
$=CHCH{=}CNO_2-$	131.55	7.47 (d, 11.2)	6.47	6.17, 2.71
$=CHCH{=}CNO_2-$	153.76			7.47
$-CH_2$-R^2	27.47	2.71 (t, 7.6)	1.60	7.47

[a] Poorly discernible in the $^1H-^{13}C$ HETCOR spectrum, identified in the HMBC spectrum.

Table 7. Selected NMR Data of Compounds 10 and 11[a]

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2-	28.20, 27.99, 27.11[b]	2.00 (m)	3.90	
$-CHNO_2CHOH$	92.76, 92.33	4.42 (m)	3.90, 2.00	
$-CHOHCH_2-$	72.94, 72.67	3.90 (m)	4.42, 2.28	2.28
$=CHOHCH_2CH{=}$	25.71	2.28 (m)	5.37	
$-CH_2CH{=}CH-$	123.15,122.71	5.37 (m)	5.54, 2.28	2.28
$-CH_2CH{=}CH-$	135.81,135.59	5.54 (m)	5.37, 2.00	2.28
$-CH_2$-R^2	28.20, 27.99, 27.11[b]	2.00 (m)	5.54	

[a] R^1, $R_2 = -(CH_2)_3CH_3$ or $-(CH_2)_6CO_2Et$. [b] Interchangeable.

Fraction IV. Chromatographic analysis revealed an intimate mixture of products difficult to separate. Repeated TLC fractionation yielded eventually a main chromatographically homogeneous band. The ^1H NMR spectrum exhibited four sets of multiplets centered at around δ 5.6, 5.4, 4.4, and 4.0, showing one-bond correlations with clusters of carbon signals at around δ 135, 123, 92, and 75, in that order. These and other data indicated that the fraction consisted of isomeric products containing a double bond and a vicinal nitrohydroxy functionality separated by a methylene group. Such an arrangement of functional groups implied four different regioisomers, each existing as two diastereoisomeric pairs of enantiomers, described by the gross structures **10/11** and **12/13**. Assignment of resonances to all possible isomers was hampered by the marked complexity of the spectra and the considerable degree of signal overlap. Nonetheless it was possible to discriminate the specific subsets of proton and carbon resonances belonging to each of the spin systems in regioisomeric structures **10**/**11** and **12**/**13**. The marked de-shielding influence of the nitro group on vicinal protons,[20] in particular, allowed to assign the signals at δ 2.61 and 2.81 to the diastereotopic allylic protons flanked by the nitro group in structures **12**/**13**, whereas the multiplet at δ 2.28 was attributed to the methylene protons proximal to the hydroxy group in the alternate regioisomers **10**/**11** (Tables 7 and 8). Furthermore, the integrated areas of the clearly distinguishable signals indicated an approximate ratio of **10**−**11**/**12**−**13** of 3:2. The other possible regioisomers, viz. structures **10** and **11**, or **12** and **13**, could not be distinguished by spectral analysis, nor could be the expected couples of diastereoisomers.

GC/MS Analysis. GC/MS data of the main products identified in fractions II−IV are given in Table 9 along

(20) Claridge, R. P.; Deeming, A. J.; Paul, N.; Tocher, D. A.; Ridd, J. H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3523−3528.

Table 8. Selected NMR Data of Compounds 12 and 13[a]

carbon	δ_C	δ_H (mult, *J*, Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
R^1-CH_2−	26.08	1.50 (m)	4.05	1.62−1.60, 1.35−1.30
−*C*HOHCHNO$_2$	72.43	4.05 (m)	4.42, 1.50	2.81, 1.50
−*C*HNO$_2$CH$_2$−	92.98	4.42 (m)	4.05, 2.81, 2.61	2.81, 2.61
=CHNO$_2$*C*H$_2$CH=	29.33	2.61 (m)	5.28, 4.42, 2.81	5.54, 5.28
		2.81 (m)	5.28, 4.42, 2.61	
−CH$_2$*C*H=CH−	122.42	5.28 (m)	2.81, 2.61, 2.00	2.81, 2.61, 2.00
−CH$_2$CH=*C*H−	135.99	5.54 (m)	5.28, 2.00	2.81, 2.61, 2.00
−CH$_2$−R^2	28.20, 27.99, 27.11	2.00 (m)	5.54	

[a] R^1, R_2 = −(CH$_2$)$_3$CH$_3$ or −(CH$_2$)$_6$CO$_2$Et.

with a plausible interpretation of the origin of the main fragmentation peaks.[11,12] Retention times were deduced by comparative scrutiny of GC/MS traces of the various fractions at different stages of purification, as guided by NMR analysis. Detectable pseudomolecular ion peaks were obtained for mononitroderivatives **2** and **3** and, to a lesser extent, **4** and **5** in the CI modalities. Dinitro derivatives **8** and **9** were identified as two close peaks giving extensive fragmentation patterns even in the CI modalities. GC/MS analysis of fraction IV containing nitro alcohols **10**−**13** revealed two main peaks, one of which gave a poorly discernible molecular ion peak at *m*/*z* 371. Although repeated analyses on individual products or isolated fractions gave fairly reproducible chromatographic elution profiles and fragmentation patterns in the EI, negative and positive ions chemical ionization (NICI and PICI) modalities, the possibility that some of the peaks arose from decomposition of the products in the injection port or on the column cannot be ruled out.

Effects of Medium, Oxygen, Nitrite Concentration, and Other Reaction Conditions on Product Stability and Distribution. To establish the nature of the early products formed in the reaction of **1** with NO_2^- in cyclohexane/1% sulfuric acid, the substrate was exposed to limited amounts of NO_2^- (e.g., 0.5 molar equiv). Careful monitoring of the reaction at various intervals of time showed that the product distribution remained unchanged for up to 18 h and displayed substantial similarity with the product distribution obtained with higher NO_2^- concentrations. In separate experiments it was found that each isolated product or fraction resisted incubation in cyclohexane/1% sulfuric acid or in acetic acid for various periods of time without suffering rearrangement or decomposition.

When the reaction of **1** with NO_2^- in cyclohexane/1% sulfuric acid was conducted under an argon atmosphere, care being taken to avoid contact with air prior to work up, the product distribution was little or not affected. This ruled out a significant role of O_2 in the NO_2^--induced nitration.

Virtually identical product distributions were obtained running the reaction of **1** with NO_2^- in different acidic systems, viz. cyclohexane/1% sulfuric acid, freshly distilled acetic acid and 1% sulfuric acid, with the lipid present as a fine emulsion (data not shown). A complete conversion of the substrate to intractable polar materials, presumably polynitrated products, was observed in TFA or in various TFA/acetic acid mixtures. In all cases examined, reddish fumes denoting NO_2 were visible in the headspace of the reaction flask.

The usual pattern of nitration products was formed in small amounts (5% overall yield) when **1** was finely suspended in the presence of NO_2^- in phosphate buffer, at pH 2.5 at 37 °C for 4 h, that is, under conditions simulating those occurring in the gastric compartment, as well as in 0.1 M sodium dodecyl sulfate (SDS) as solubilizing agent (TLC evidence). In both cases, the extent of substrate consumption was limited, not exceeding 10%.

Despite careful chromatographic analysis, we failed to identify in the reaction mixtures 1,2-dinitro derivatives, 1,2-nitronitrates or the conjugated 5-nitro-1,3-diene products recently hypothesized.[8,9] Likewise, products deriving from intramolecular cyclizations and/or intermolecular

Table 9. GC/MS Data of Compounds 2−13 with Possible Interpretations of Main Fragmentation Routes

compd	t_R (min)	EI, *m*/*z* [fragment]	PICI	NICI
2	19.6	336 (10) $[M - OH]^+$, 308 (27) $[M - OEt]^+$, 196 (100) $[M - C_6H_{12}COOEt]^+$, 107 (100) $[C_8H_{11}]^+$	354 $(M + H)^+$, 382 $(M + C_2H_5)^+$	353
3	19.5	336 (27) $[M - OH]^+$, 250 (100) $[M - NO_2 - C_4H_9]^+$, 121 (95) $[C_9H_{13}]^+$, 107 (100) $[C_8H_{11}]^+$	354 $(M + H)^+$, 382 $(M + C_2H_5)^+$	353
4/5	10.3	308 (1) $[M - OEt]^+$, 158 (30) $[(CH_2)_6COOEt + H]^+$, 143 (100) $[(CH_2)_5COOEt]^+$, 115 (100) $[(CH_2)_3COOEt]^+$	354	353
	10.7	265 (1) $[M - OEt - C_3H_7]^+$, 172 (20) $[(CH_2)_7COOEt + H]^+$, 157 (80) $[(CH_2)_6COOEt]^+$, 155 (80) $[(CH_2)_6COOEt - H_2]^+$, 111 (100) $[C_8H_{15}]^+$	354	353
6/7	18.3	353 (5) $[M]^+$, 308 (20) $[M - OEt]^+$, 259 (50) $[M - OEt - HNO_2 - H_2]^+$, 241 (40) $[M - C_8H_{16}]^+$, 217 (100) $[M - OEt - HNO_2 - H_2 - C_3H_6]^+$	354	353
8	13.6	308 (100) $[M + H - NO_2 - OEt]^+$, 262 (70) $[M + H - 2NO_2 - OEt]^+$		398
9	14.0	308 (40) $[M + H - NO_2 - OEt]^+$, 262 (80) $[M + H - 2NO_2 - OEt]^+$, 164 (100) $[M - (CH_2)_7COOEt - OH - NO_2]^+$		
10/13	12.3	229 (2) $[M - C_4H_7COOEt]^+$, 196 (80) $[M - C_6H_{12}COOEt - H_2O]^+$, 194 (100) $[M - C_6H_{12}COOEt - H_2O - H_2]^+$, 166 (60) $[M - C_8H_{16}COOEt - H_2O - H_2]^+$		
	13.3	371 (1) $[M]^+$, 254 (20) $[M - C_3H_6COOEt - H_2]^+$, 226 (100) $[M - C_5H_{10}COOEt - H_2]^+$, 210 (30) $[M - C_5H_{10}COOEt - H_2O]^+$, 178 (35) $[M - C_4H_8COOEt - NO_2]^+$,		

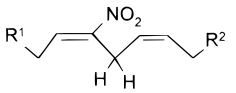
cross-linking processes (e.g., dimerization) were apparently absent.

Acid-Promoted Reaction of Methyl Oleate with NO_2^-. In another series of experiments we extended the investigation to a simpler unsaturated fatty acid derivative, methyl oleate (methyl (*Z*)-octadec-9-enoate) to gain more information on the observed nitration reactions. Under conditions similar to those adopted for **1**, the reaction afforded a main fraction positive to Griess reagent. This consisted of an intimate mixture of nitration products difficult to separate by repeated TLC and appearing as a broad peak on GC MS analysis. The products could be readily identified as **14/15** and the allylic nitroderivatives **16/17**. By ^{1}H NMR analysis, an approximate formation ratio of (**14** + **15**)/(**16** + **17**) of 1:1 could be estimated, which did not change during the course of the reaction. When isolated, products **14**−**17** proved fairly stable under the reaction conditions for sufficiently prolonged periods of time.

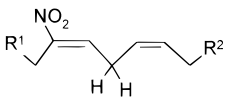
Mechanistic Issues. Considering the main equilibria underlying the acid-promoted conversion of NO_2^- to HNO_2 and its subsequent decomposition[21,22] (eqs 1−4), products **2**−**13** could be envisaged as originating from at least three different processes, namely: (i) a free radical attack of NO_2; (ii) the addition of NO^+ (or NO_2^+); and (iii) a direct interaction with N_2O_3 or N_2O_4.

$$NO_2^- + H^+ \rightleftharpoons HNO_2$$
$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$
$$N_2O_3 \rightleftharpoons NO + NO_2$$
$$2NO + O_2 \longrightarrow 2NO_2$$

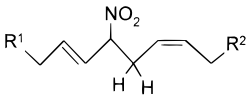
A series of separate experiments was thus performed in an attempt to differentiate between radical and ionic nitration pathways. NO_2^- was allowed to decompose in a separate flask in 1% sulfuric acid and the nitrogen oxides that evolved were passed through a solution of **1** in anhydrous cyclohexane. TLC analysis showed a pattern of products virtually identical to that obtained in the biphasic cyclohexane/1% sulfuric acid system, comprising all of the products **2**−**13**. The same results were obtained by slowly bubbling purified NO into an air-equilibrated cyclohexane/1% sulfuric acid system containing **1** but no NO_2^-. In contrast, reaction of **1** with NO_2BF_4 (containing some NO^+) in chloroform gave small amounts of nitration products, consistent with a recent report,[9] but TLC analysis ruled out significant analogies with the product pattern obtained from HNO_2-derived nitrogen oxides. The reaction of **1** with NO_2^- in cyclohexane/1% sulfuric acid was also carried out in the presence of 1,3-dinitrobenzene (10 molar equiv) to eliminate radical reactions and provide an opportunity for an ionic reaction to occur.[23] Under such conditions, product formation was not inhibited to any significant extent. This, however, may be due to a substantial failure of 1,3-dinitrobenzene to react with HNO_2-derived species, as evidenced in control experiments in which the compound was recovered unchanged after prolonged exposure to a 10-fold excess of NO_2^- in the biphasic system.



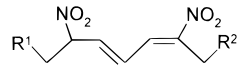
2: R^1= -$(CH_2)_6CO_2Et$, R^2= -$(CH_2)_3CH_3$
3: R^1= -$(CH_2)_3CH_3$, R^2=-$(CH_2)_6CO_2Et$



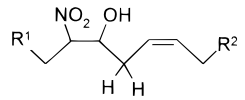
4: R^1 = -$(CH_2)_6CO_2Et$, R^2 = -$(CH_2)_3CH_3$
5: R^1= -$(CH_2)_3CH_3$, R^2= -$(CH_2)_6CO_2Et$



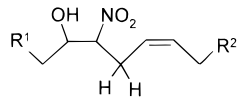
6: R^1= -$(CH_2)_5CO_2Et$, R^2 = -$(CH_2)_3CH_3$
7: R^1= -$(CH_2)_2CH_3$, R^2 = -$(CH_2)_6CO_2Et$



8: R^1=-$(CH_2)_3CH_3$, R^2= -$(CH_2)_6CO_2Et$
9: R^1-$(CH_2)_6CO_2Et$,R^2 = -$(CH_2)_3CH_3$



10: R^1= -$(CH_2)_6CO_2Et$, R^2 = -$(CH_2)_3CH_3$
11: R^1= -$(CH_2)_3CH_3$, R^2 = -$(CH_2)_6CO_2Et$

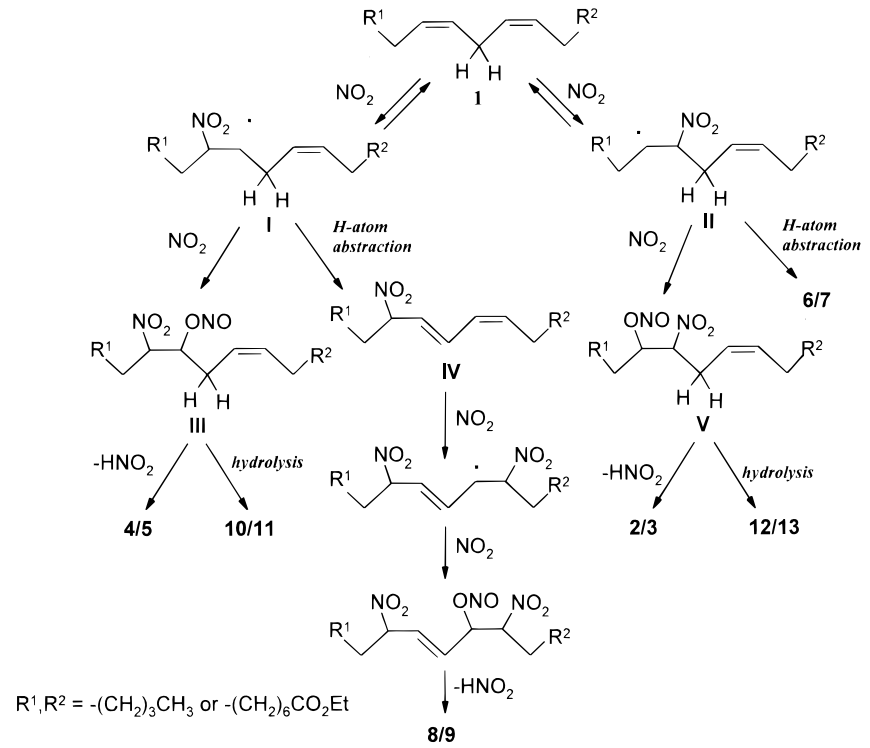


12: R^1= -$(CH_2)_6CO_2Et$, R^2= -$(CH_2)_3CH_3$
13: R^1 = -$(CH_2)_3CH_3$, R^2= -$(CH_2)_6CO_2Et$

On these bases, it was argued that gaseous nitrogen oxides, viz. NO, NO_2, N_2O_3 and/or N_2O_4, played an important role in the HNO_2-dependent nitration of **1**.[21] The lack of detectable formation of deeply colored nitroso compounds, generally regarded as a "footprint" of N_2O_3 and N_2O_4,[24−26] the known reluctance of NO to add to double bonds,[27] the apparent analogy of most of the nitration products with those arising by homolytic attack of NO_2 to alkenes,[25,28−30] and the visible generation of NO_2 in the reaction media further allowed to envisage NO_2 as a main nitrating species involved in the formation of products **2**−**13**. The NO_2^--based mechanism depicted in Scheme 1 envisages two independent pathways of evolution of the primary β-nitroalkyl radical intermediates, namely (a) free radical combination with another molecule of NO_2 and (b) β H-atom abstraction.

(21) Beake, B. D.; Costantine, J.; Moodie, R. B. *J. Chem. Soc., Perkin Trans. 2* **1994**, 335−340.
(22) Kono, Y.; Shibata, H.; Kodama, Y.; Sawa, Y. *Biochem. J.* **1995**, *312*, 947−953.
(23) We acknowledge suggestion of this experiment by one of the referees.
(24) Birchall, J. M.; Bloom, A. J.; Haszeldine, R. N.; Willis, C. J. *J. Chem. Soc.* **1962,** 3021−3032.
(25) Golding, P.; Powell, J. L.; Ridd, J. H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 813−819.
(26) Brindley, P. B.; Nicholson, S. H. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1808−1813.
(27) Park, J. S. B.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2579−2583.
(28) Hata, E., Yamada, T., Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 3629−3636.
(29) Chatterjee, J.; Coombes, R. G.; Barnes, J. R.; Fildes, M. J. *J. Chem. Soc., Perkin Trans. 2* **1995,** 1031−1032.
(30) Kelly, D. R.; Jones, S.; Adigun, J. O.; Koh, K. S. V.; Hibbs, D. E.; Hursthouse, M. B.; Jackson, S. K. *Tetrahedron* **1997**, *53*, 17221−17234.

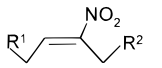
Scheme 1



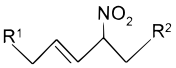
Pathway (a) would provide a plausible route to **2**−**5** via acid-promoted loss of HNO_2 from labile 1,2-nitronitrite intermediates.[25,28] Structures **6**/**7** containing the 3-nitro-1,5-hexadiene moiety exemplify the products putatively arising from pathway (b), *viz.* β-hydrogen atom abstraction from the nitroalkyl radicals **II**, a mechanism supported by recent literature evidence.[25] H-atom abstraction is favored at position γ rather than α to the nitro group by both statistical (CH_2 vs CH) and steric factors, which apparently play a more decisive role than the thermodynamic gain derived from conjugation of the incoming double bond to the nitro group. Alternate heterolytic routes to **6**/**7** can also be envisaged, e.g., via attack of NO_2^+ to the 1,4-pentadiene moiety of **1**, or from loss of HNO_2 from the nitronitrite intermediates **V**. These however seem less probable considering the expectedly preferential elimination by loss of the more acidic proton α to the nitro group.

Operation of an H-atom abstraction mechanism on the nitroalkyl radicals **I**, precursors to **4** and **5**, would conceivably account for the generation of the dinitro derivatives **8** and **9**. In this case, the primary products would be either of the possible 5-nitro-1,3-diene products **IV** [8] which would be susceptible to homolytic attack by NO_2 to form a resonance-stabilized allylic radical. Compliance to the generally held hydrolytic origin of nitro alcohols from nitronitrite and/or nitronitrate intermediates[25] would suggest formation of **10**/**11** and **12**/**13** from the nitronitrite intermediates **V** and **III**, respectively.

The proposed mechanism would be in accord also with the results of the experiments carried out on methyl oleate, in which the presence of a single double bond prevented several of the complications derived from the skipped diene functionality of **1**. The identification of products **14**−**17** corroborated the existence of two main evolution routes of primary nitrated intermediates, leading to nitroalkene and allylic nitro functionalities. In all cases, the lack of appreciable changes in the product distribution with time and NO_2^- concentration, and the absolute stability of **2**−**13** under the reaction conditions indicated that the isolated species are end products of competing nitration pathways which may be under kinetic control.



14: R^1= -$(CH_2)_6CO_2Me$, R^2 = -$(CH_2)_6CH_3$
15: R^1= -$(CH_2)_6CH_3$, R^2 = -$(CH_2)_6CO_2Me$



16: R^1 = -$(CH_2)_5CO_2Me$, R^2 = -$(CH_2)_6CH_3$
17: R^1= -$(CH_2)_5CH_3$, R^2= -$(CH_2)_6CO_2Me$

Of course, alternate reaction pathways, e.g., ionic routes and/or nitrosation processes, cannot be ruled out based on the available evidence. These pathways are not mutually exclusive and may well concur to afford at least some of the isolated products.

Preliminary Biological Experiments. In preliminary experiments some of the main nitration products of **1**, namely pure **2** and a fraction containing **3**−**7**, were evaluated for their toxicity against a human keratinocyte cell line. This cell line is commonly used for screening potentially cytotoxic substances of dermatological relevance as well as environmental toxicants. At doses of 0.1−10 μg/10^4 cells none of the isolated products exerted significant toxic effects after 24 and 48 h incubation times.

Conclusions

The present study provides an inventory of the main products formed by reaction of the model polyunsaturated fatty acid derivative **1** with NO_2^- in acidic media. Salient

outcomes include the first complete structural characterization of *single* nitration products of unsaturated fatty acids and the provision of a fully documented library of NMR data for such products. Although intrinsically limited in scope, because of the complexity of product mixtures and the poor degree of selectivity, the acid-promoted reaction of NO_2^- with polyunsaturated fatty acids may represent a convenient and straightforward route for the small scale preparation of nitrated lipid products of potential biological and dermocosmetic interest. The products described in this paper provide also a chemical background to assess the possible structural modifications caused by high NO_2^- levels on lipid components under acidic conditions of (patho)physiological relevance, and to elucidate the mechanisms of the inhibitory effects of unsaturated fatty acids on toxic nitrosamine formation induced by HNO_2.[13]

Experimental Section

General Methods. Ethyl linoleate (**1**, 98%), methyl oleate (99%), nitronium tetrafluoborate (85% containing nitrosonium tetrafluoroborate), and 1,3-dinitrobenzene were used as obtained. NO gas (electronic grade, 99.99%) was purified from higher nitrogen oxides by passage through a solution of concentrated NaOH previously purged with argon for 1 h. CAUTION! Nitrogen oxides are highly toxic and all operations must be carried out under an efficient hood.

GC−MS was carried out on a GC instrument coupled with a quadrupole mass spectrometer using a 60 m crossbond 5% diphenyl-95% dimethylpolysiloxane column (0.25 mm i.d., 0.25 μ df). Helium was the carrier gas. CI-MS measurements were carried out using methane as the reagent gas. The temperature program of the column was as follows: at 60 °C, hold time = 2 min; from 60 to 260 °C, rate = 40 °C/min; from 260 °C to 280 °C, rate = 10 °C/min. The EI spectra were obtained at 70 eV.

UV and IR spectra were performed using a diode array and a FT-IR spectrophotometer, respectively. ^{1}H (^{13}C) NMR spectra (TMS as internal standard) were recorded at 400 (100) MHz. Hartmann−Hahn, ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR and ^{1}H−^{13}C HMBC NMR experiments were run at 400.1 MHz using standard pulse programs. For all isolated nitration products of **1,** resonances due to $-OCH_2-$, OCH_2CH_3, C-2 (CH_2), C-18 (CH_3), C-3/C-7/C-15 (CH_2) and C-4/C-5/C-6/C-16/C-17 (CH_2) groups appear in the ^{1}H/^{13}C NMR spectra at δ ($CDCl_3$) 4.11 (q, J = 7.2 Hz)/60.98; 1.25 (t, J = 7.2 Hz)/15.03; 2.28 (t, J = 7.6)/35.07; 0.90 (m)/14.80; 1.50−1.62 (m)/29.9−25.6, 1.35/32.3−29.7, in that order. Accordingly, these resonances were omitted in the tables reporting selected NMR data. Analytical and preparative TLC analyses were performed on F254 0.25 and 0.5 mm silica gel plates, high performance TLC (HPTLC) or Ag-impregnated plates using cyclohexanes−ethyl acetate 95:5 as the eluant unless otherwise stated. Silver nitrate impregnated silica gel plates were prepared as described.[31] Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid),[15] potassium dichromate in 20% sulfuric acid and iodine were used for product detection on TLC plates. Flash chromatography was performed using 270−400 mesh silica gel. Product purity was determined by determining percentage proportions of main components from integrated resonances in the ^{1}H NMR spectra, taking the $-OCH_2$ quartet at δ 4.11 (2H) as internal reference.[11,12] Molecular mechanics (MM+) calculations were carried out with Hyperchem 5.0 package.

Reaction of Unsaturated Fatty Acids with Nitrite. To a solution of **1** or methyl oleate in cyclohexane (0.10 M), 1% sulfuric acid (1:1 v/v with respect to the organic layer) was added followed by sodium nitrite in two portions at 15 min intervals (0.50 M final concentration) while the biphasic system was taken under vigorous stirring in a stoppered round-bottom flask at room temperature. After 40 min, the organic layer was separated, washed with brine and dried over sodium sulfate to give a yellow residue which was analyzed as described below. When required, the reaction was carried out with purging of the biphasic system with argon for at least 30 min prior to addition of a deareated solution of nitrite in water (1 g/mL). The reaction mixture was neutralized by addition of a solution of $KHCO_3$ in argon atmosphere before work up as above.

Other reaction conditions were as follows: a fine emulsion of **1** in 1% sulfuric acid (30 mg/mL) was reacted with nitrite added portionwise for 40 min at room temperature, and then was extracted with cyclohexane for product analysis as above; (ii) as in (i) using 0.1 M phosphate buffer, pH 2.5 containing 10% acetonitrile as reaction medium at 37 °C, and a reaction time of 18−24 h; (iii) **1** was dissolved in glacial acetic acid (0.1 M), and was added with nitrite (0.5 M) under vigorous stirring at room temperature. After 1 h the mixture was diluted with water and extracted twice with cyclohexane; (iv) as in (iii) using as solvent pure TFA or TFA/AcOH mixtures in different proportions from 50:50 to 5:95 v/v, at room temperature and a reaction time of 30 min; (v) a solution of **1** (2.5 mM) was prepared in 0.1 M SDS and 0.1 M phosphate buffer, pH 2.5 thermostated at 37 °C, and nitrite (5 molar equiv) was added portionwise as described above.

Reaction of 1 with NO_2BF_4. The reaction was carried out as described[9] with modifications. In brief, a solution of **1** (56 mg, 0.18 mmol) in acid-free dry chloroform (50 mL) was purged with argon and added with solid NO_2BF_4 (0.36 mmol). The mixture was taken under argon atmosphere overnight at room temperature, and then was treated with a small volume of 0.1 M phosphate buffer, pH 7.4. The organic layer was separated, washed with brine and dried over sodium sulfate.

Reaction of 1 with NO and Other Nitric Oxides. Purified NO gas was slowly bubbled for about 15 min into a vigorously stirred solution of **1** (308 mg, 1 mmol) in air equilibrated cyclohexane (25 mL). The solution was then flushed with argon for 10 min and the solvent was evaporated to dryness. In other experiments, a solution of nitrite (860 mg) in water (2 mL) was added to 10% sulfuric acid over 10 min. The red-orange gas which developed was conveyed with a flux of argon into a solution of **1** in dry cyclohexane (0.05 M). Fifteen minutes after the development of the red fumes had completed, the reaction mixture was washed with brine and the organic layer was dried over sodium sulfate and evaporated to dryness.

Isolation of Compounds 2−13. For preparative purposes, reaction of **1** with nitrite was carried out in cyclohexane/1% sulfuric acid as described above using 2.0 g of the starting material. After work up of the reaction mixture, the yellowish residue obtained was fractionated by flash chromatography (2.5 × 45 cm column) using cyclohexanes−ethyl acetate (19:1 to 4:1, gradient mixtures) to afford five main fractions. Fraction I (600 mg, R_f = 0.7 cyclohexanes−ethyl acetate 95:5) consisting of almost pure **1** and fraction V (26 mg, R_f = 0.1) were not further purified. Fraction II (215 mg, R_f = 0.6−0.45) was fractionated by PLC using cyclohexanes−ethyl acetate 95:5 as the eluant to give two main bands, IIa (63 mg, R_f = 0.60−0.50) and IIb (44 mg, R_f = 0.50−0.45), which were further purified on Ag-impregnated silica gel plates using the same eluant. Two main fractions were obtained from IIa, namely fraction IIa′ (10 mg, R_f = 0.57−0.50) which consisted mainly of compounds **4**/**5** and **3** (NMR evidence), and fraction IIa″ (27 mg, R_f = 0.50−0.47), containing compound **2** and **6**/**7**. Further purification of fraction IIa′ on PLC plates gave pure **3** (5 mg, R_f = 0.53). PLC purification of the IIb fraction afforded pure **2** (25 mg, R_f = 0.47). Fraction III (28 mg, R_f = 0.4−0.35) was purified on PLC plates to give two main bands IIIa (12 mg, R_f = 0.40) and IIIb (12 mg, R_f = 0.36) which on further fractionation on HPTLC plates gave fractions IIIa′ and IIIb′ consisting mainly of compound **8** (9 mg, R_f = 0.40) and compound **9** (10 mg, R_f=0.36), respectively. Fraction IV (86 mg, R_f = 0.30−0.15) was subjected to two fractionation steps

(31) Morris, L. J.; Wharry, D. M.; Hammond, E. W. *J. Chromatogr.* **1967**, *31*, 69−76.

on PLC plates using cyclohexanes−ethyl acetate 95:5 and 90:10 as the eluant to give eventually a main fraction consisting of compounds **10**/**11** and **12**/**13** (20 mg, R_f 0.15).

Isolation of Compounds 14−17. Reaction of methyl oleate (0.3 g) with nitrite was carried out in cyclohexane/1% sulfuric acid as above. After workup of the reaction mixture, the yellowish residue obtained was chromatographed on silica gel plates using cyclohexanes−ethyl acetate 90:10 as the eluant to give a main band (35 mg, R_f = 0.7 cyclohexanes−ethyl acetate 90:10).

Ethyl (9*E*,12*Z*)-10-nitrooctadeca-9,12-dienoate (2): UV λ_{max} (cyclohexane) 235 nm; FT-IR ($CHCl_3$) ν_{max} 1724, 1602, 1520, 1465, 1375, 1337 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; GC MS, see Table 9.

Ethyl (9*Z*,12*E*)-12-nitrooctadeca-9,12-dienoate (3): UV λ_{max} (cyclohexane) 235 nm; FT-IR ($CHCl_3$) ν_{max} 1724, 1602, 1520, 1466, 1375, 1337 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 2; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 2; GC MS, see Table 9.

Ethyl (9*E*,12*Z*)-9-nitrooctadeca-9,12-dienoate (4) and ethyl (9*Z*,12*E*)-13-nitrooctadeca-9,12-dienoate (5): ^1H NMR (400 MHz, $CDCl_3$), see Table 3; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 3; GC MS, see Table 9.

Ethyl (8*E*,12*Z*)-10-nitrooctadeca-8,12-dienoate (6) and ethyl (9*Z*,13*E*)-12-nitrooctadeca-9,13-dienoate (7): ^1H NMR (400 MHz, $CDCl_3$), see Table 4; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 4; GC MS, see Table 9.

Ethyl (9*E*,11*E*)-9,13-dinitrooctadeca-9,11-dienoate (8) UV λ_{max} (cyclohexane) 284 nm; FT-IR ($CHCl_3$) ν_{max} 1724, 1602, 1554, 1520, 1375, 1328 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 5; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 5; GC MS: see Table 9.

Ethyl (10*E*,12*E*)-9,13-dinitrooctadeca-10,12-dienoate (9): UV λ_{max} (cyclohexane) 284 nm; FT-IR ($CHCl_3$) ν_{max} 1724, 1636, 1602, 1554, 1520, 1466, 1375, 1328, 1277 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Table 6; ^{13}C NMR (100 MHz, $CDCl_3$), see Table 6; GC MS: See Table 9.

Ethyl (12*Z*)-10-hydroxy-9-nitro-12-octadecenoate (10), ethyl (9*Z*)-12-hydroxy-13-nitro-9-octadecenoate (11), ethyl (12*Z*)-9-hydroxy-10-nitro-12-octadecenoate (12) and ethyl (9*Z*)-13-hydroxy-12-nitro-9-octadecenoate (13): FT-IR ($CHCl_3$) ν_{max} 1724, 1602, 1550, 1450, 1375, 1328 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), see Tables 7 and 8; ^{13}C NMR (100 MHz, $CDCl_3$), see Tables 7 and 8; GC−MS See Table 9.

Methyl (*E*)-10-nitrooctadec-9-enoate (14) or methyl (*E*)-9-nitrooctadec-9-enoate (15) and methyl (*E*)-10-nitrooctadec-8-enoate (16) or methyl (*E*)-9-nitrooctadec-10-enoate (17): UV λ_{max} (cyclohexane) 240 nm; FT-IR ($CHCl_3$) ν_{max} 1720, 1649, 1550, 1520, 1466, 1375, 1272 cm^{-1}; $^1H/^{13}C$ NMR (400 MHz, $CDCl_3$ selected resonances), δ (ppm): 2.19/29.7 (**14**/**15**, $CH_2CH{=}CNO_2$), 7.07/137.2 ($CH_2CH{=}CNO_2$), 152.6 ($CH{=}CNO_2CH_2$), 2.56/27.9 ($CH{=}CNO_2CH_2$); 2.10/32.25 (**16**/**17**, $CH_2CH{=}CH$), 5.84/139.8 ($CH_2CH{=}CH$), 5.61/125.27 ($CH{=}CHCHNO_2$), 4.84/90.92 ($=CHCHNO_2CH_2$), 2.15−1.96/34.88 ($=CHCHNO_2CH_2$); GC−MS t_R 15.60−16.10 min (broad peak); EI *m*/*z* 341 (M^+, 5), 324 (M − OH, 80), 282 (M − OH − C_3H_7, 100).

Biological Experiments. Human keratinocytes from HaCat cell line were grown in Dulbecco modified Eagle's medium and seeded in 24 well plates (1×10^4 per well). To test the effect of nitration products of **1**, 0.1, 1, and 10 μg amounts dissolved in ethanol were added to the medium. The control cells were treated with 95% ethanol. All the assays were conducted in triplicate. The viability of cultured cells was determined at 24 and 48 h using an adapted 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) eluted stain assay.[32] The effect of the tested substances on the treated cells was expressed as percentage of control cell viability.

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Supporting Information Available: ^1H NMR and ^1H-H COSY spectra of compounds **2**, **3**, **8**, and **9** as well as of fractions containing compounds **4**/**5**, **6**/**7**, and **10**−**13.** Spectra of Hartmann−Hahn experiments for compounds **2** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(32) Denizot, F.; Lang, R. *J. Immunol. Met.* **1986**, *89*, 271−277.