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The acid-promoted reaction of ethyl linoleate with nitrite. New insights from ^{15}N-labelling and peculiar reactivity of a model skipped diene

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Dedicated in the memory of Professor Guido Sodano

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Abstract—The acid-promoted reaction of ethyl linoleate with nitrite ions was re-examined by an integrated approach based on the use of $^{15}NO_2^-$ combined with extensive GC–MS (EI, NICI, PICI) and 2D ^1H,^{15}N and ^1H,^{13}C NMR analysis. The less polar products proved to be regioisomeric *E*-nitroalkenes, novel *Z*-nitroalkenes, and 3-nitro-1,5-hexadienes derivatives. A medium polarity fraction consisted mainly of stereo- and regioisomeric 1,2-nitronitrates along with 1,5-dinitro-1,3-pentadiene compounds. Novel 5-nitro-2,4-pentadienone products could be identified in the most polar fraction, which featured 1,2-nitroalcohols as the most abundant components. Under similar conditions 1,4-hexadiene gave mainly a nitrofuroxan derivative.

1. Introduction

The acid-promoted reaction of polyunsaturated fatty acids with nitrite ions is a potential mechanism of membrane modification that may affect cellular permeability in the gastric compartment (pH 2.5–4.5), following cerebral ischemia and reperfusion, in certain inflammatory reactions of the skin and in all (patho)physiological conditions associated with acidosis and nitrite accumulation.[1] Despite the biomedical relevance and the intrinsic chemical interest of diene nitrosation/nitration,[2–8] several aspects of this reaction have remained so far elusive, because of the many concurrent pathways and the complex mixtures of products. Literature data on the acid promoted reactivity of simple diene substrates with nitrite are also surprisingly scanty.

Recently, in the course of a systematic investigation of the acid-induced reaction of unsaturated lipids with nitrite ions, we undertook a detailed investigation of the reactivity of ethyl linoleate, the simplest polyunsaturated fatty acid derivative. Following extensive chromatographic fractionations, some prominent nitration products could eventually be isolated and spectroscopically characterised. These included isomeric *E*-nitroalkene, 1,2-nitrohydroxyl, 3-nitro-1,5-hexadiene and 1,5-dinitro-1,3-pentadiene derivatives.[9]

However, a complete picture of the reaction products was hampered by the marked complexity of the mixtures and the failure of conventional chromatographic techniques to afford sufficiently purified fractions amenable to 2D ^1H,^1H and ^1H,^{13}C NMR correlation analysis; in consequence, several aspects of the underlying chemistry concerning the reactivity of the skipped 1,4-diene system with nitrous acid and species thereof could not be elucidated.

In this paper we exploit ^{15}N-labelling to trace the fate of nitrogen in the acid-promoted reaction of nitrite with ethyl linoleate, and the potential of a combined GC–MS/NMR analysis for the in toto characterisation of the reaction mixture without making recourse to extensive chromatographic fractionations. The overall pattern of reactivity was then compared with that of 1,4-hexadiene as a model skipped diene.

2. Results and discussion

2.1. Reaction of ethyl linoleate with acidic nitrite

The reaction of ethyl linoleate with $^{15}NO_2^-$ was carried out

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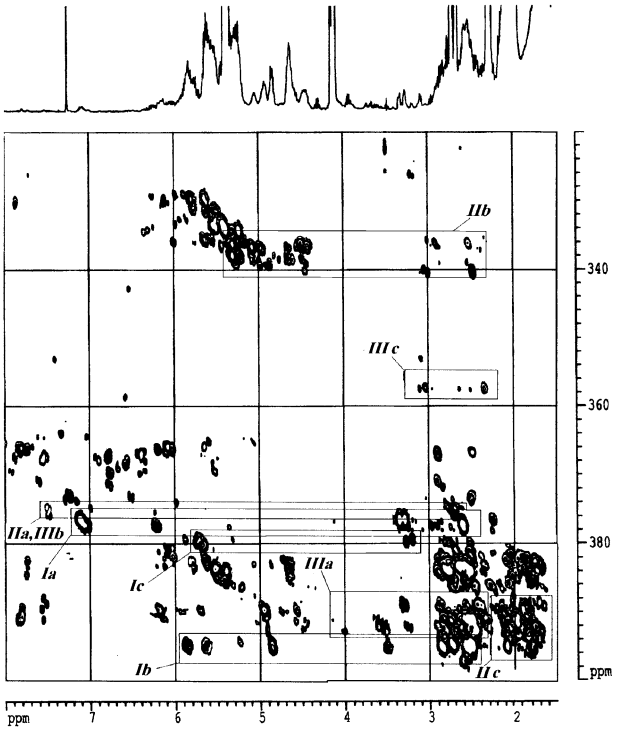


Figure 1. $^{1}H-^{15}N$ HMBC spectrum of the cyclohexane extractable mixture obtained by acid-promoted reaction of ethyl linoleate with [^{15}N]$NaNO_2$. Letters denote regions of the spectum comprising cross peaks due to groups of components identified in fractions I–III. All highlighted correlations were more clearly apparent in the $^{1}H-^{15}N$ HMBC spectra of the separated fractions (not shown).

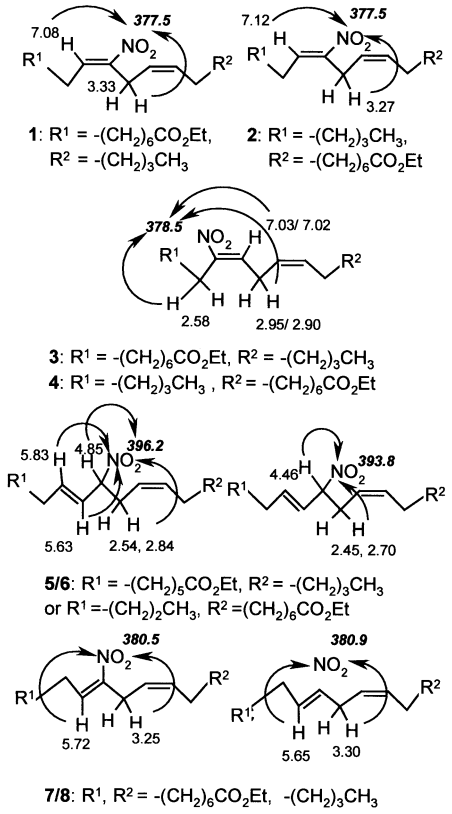


Figure 2. Structures and ^{15}N resonances assignments of the main components of fraction I from the reaction mixture of ethyl linoleate with [^{15}N]$NaNO_2$. Arrows indicate multiple bond $^{1}H-^{15}N$ correlations.

in 1% sulfuric acid[9] using 4 M equiv. of nitrite with respect to the substrate. After 3 h, when linoleate consumption was about 50% (^{1}H NMR evidence) TLC analysis indicated formation of a collection of products positive to the Griess reagent for species with nitrosating properties.

Fig. 1 shows the $^{1}H,^{15}N$ HMBC spectrum of the whole reaction mixture. Apparently, ^{15}N signals fell for the most part in three regions at δ 330–340, 365–375 and 390–395. To ease identification of specific $^{1}H-^{15}N$ correlations, the mixture was chromatographed on silica gel plates to afford three main UV- and Griess-positive fractions that were subjected to $^{1}H,^{13}C,^{15}N$ NMR and GC–MS analysis.

For comparative purposes, the same data were collected from analogous fractions obtained by reaction with unlabelled nitrite.

2.2. Fraction I

The less polar fraction (fraction I, R_f 0.45–0.60, cyclohexane/ethyl acetate 95:5) showed as main feature a distinct group of ^{15}N signals at δ 376–381 correlating with proton resonances spanning from ca. δ 7.0 to 2.0 (region Ia of Fig. 1). In this fraction, correlation peaks ascribable to the four regioisomeric *E*-nitroalkene derivatives **1**–**4** could readily be identified (Fig. 2).

A characteristic pattern of cross peaks denoting coupling between a nitrogen signal at δ 396.2 and proton resonances at δ 5.83, 5.63 and 4.85 was also apparent (region Ib of Fig. 1). This was ascribed to a 3-nitro-1,5-hexadiene (**5**). Another noticeable feature was a proton signal at δ 4.46 showing one-bond correlation with a carbon resonance at δ 89.1 and with protons resonating at δ 2.70 and 2.45 in the COSY spectrum. In the $^{1}H,^{15}N$ HMBC spectrum cross peaks between proton resonances at δ 4.46, 2.70 and 2.45 and a nitrogen signal at δ 393.8 were well discernible. Comparison of the proton–carbon correlations with those of **5** allowed formulation of the compound as the regioisomer **6** (Fig. 2).

Well discernible cross peaks were also observed between a nitrogen at δ 380.5 and protons at δ 5.72 and 3.25; and between a nitrogen at δ 380.9 and protons at δ 5.65 and 3.30 (region Ic of Fig. 1). These data argued for regioisomeric *Z*-nitroalkenes **7** and **8**, in which the *Z* geometry of the double bond hindered the β alkene proton from the deshielding influence of the nitro group.[10]

GC–MS analysis (EI, PICI, NICI) of the fraction allowed straightforward assignment of the fragmentation patterns of regioisomeric nitroalkenes. These eluted under the peaks at R_t 47.25–47.50 min and gave detectable (pseudo)molecular ion peaks in the PICI (*m/z* 355 $(M+H)^+$, 383 $(M+C_2H_5)^+$) and NICI (*m/z* 354) spectra of the ^{15}N-labelled fraction. Different fragmentation patterns for isomeric 10- and 12-nitro isomers **1** and **2** (R_t 47.49 and 47.41 min, respectively) were noted (Fig. 3). Such difference would denote a destabilizing effect of the nitro group toward adjacent allylic fragmentation of the skipped diene moiety.

Regioisomeric **5/6** eluted as a single GC peak at R_t

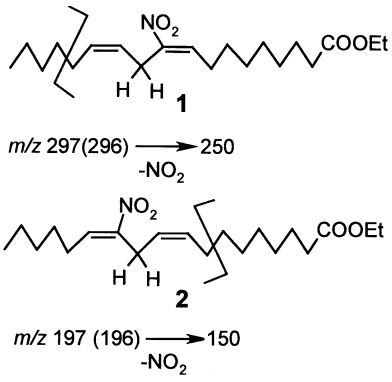


Figure 3. Fragmentation patterns of labelled (unlabelled) nitroalkenes **1** and **2**.

46.02 min, and showed pseudomolecular ion peaks in the PICI spectrum at *m/z* 355 $(M+H)^+$. Intense fragmentation peaks at *m/z* 309/306 and 261 were apparent in the EI/MS spectrum, due to losses of the OEt radical and/or of labelled $H^{15}NO_2$ as well as peaks at *m/z* 243 and 217 which secured identification in comparison with the fragmentation pattern obtained from unlabelled compounds.[9]

2.3. Fraction II

The $^1H,^{15}N$ HMBC spectrum of fraction II displayed cross-peaks pertaining to the dinitrodiene derivatives **9/10**, correlating the proton signal(s) at δ 7.47/7.48 and 2.69/2.71 with two nitrogen resonances at δ 376.8/377.0 (Region IIa, Fig. 1). Less intense but discernible cross peaks were also observed between protons signals in the δ 1.75–1.80 region and the nitrogen resonance at δ 396 for the allylic NO_2 group. Scalar coupling of the protons at δ 7.47/7.48 (J=3.6 Hz) and 6.18 (J=1.2 Hz) with the ^{15}N nitrogens was well apparent in the ^1H NMR spectrum.

Other typical features of the spectrum were: (a) a group of ^{15}N resonances at δ 335–340 correlating with protons resonating between δ 4.4 and 5.6 (Region IIb, Fig. 1); and (b) a group of cross peaks correlating nitrogen signals at δ 390–395 with protons resonating at δ around 2.0 and 2.6 (Region IIc, Fig. 1). On the basis of $^1H,^1H$ COSY, $^1H,^{13}C$ HMQC and HMBC experiments, these products could be assigned the structures of regio- and stereoisomeric 1,2-nitronitrate derivatives of the type **11/12** (Fig. 4).[5]

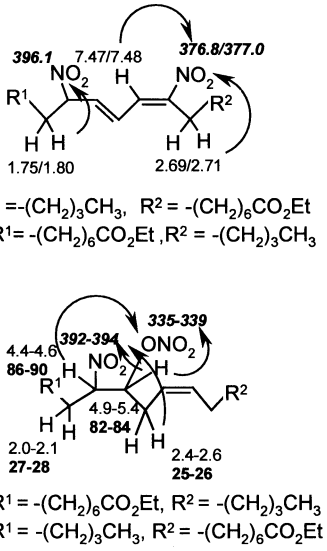


Figure 4. Structures and ^{15}N (italicized), ^{13}C (enbolded) and ^1H resonances of the main components of fraction II. Arrows indicate ^1H–^{15}N correlations.

GC–MS analysis of the fraction showed cluster of peaks eluting at R_t 44.7 min which were identified as **9/10** based on the pseudomolecular ion peaks at *m/z* 401 in the PICI spectrum and the peculiar fragments in the EI spectrum at *m/z* 353 ($M-{}^{15}NO_2$), 305 ($M-H^{15}NO_2-{}^{15}NO_2$). Another group of peaks eluting at R_t 51.0 min and exhibiting pseudomolecular ion peaks in the NICI spectrum at *m/z* 418 and a prominent fragmentaton peak at *m/z* 370 for the loss of labelled HNO_2 were identified as isomeric nitronitrates.

2.4. Fraction III

The $^1H,^{15}N$ HMBC spectrum of fraction III displayed correlation peaks for isomeric 1,2-nitroalcohols **13/14** and **15/16** (Fig. 5).

GC–MS analysis of the fraction revealed a cluster of peaks at R_t 45.9 min which gave pseudomolecular ion peaks in the PICI (*m/z* 373) and NICI modalities (*m/z* 372) and EI fragmentation peaks at *m/z* 325 and 307 due to sequential loss of $^{15}NO_2$ and H_2O, and as well as at *m/z* 255 and 227 (*m/z* 254 and 226 in the unlabelled fraction).[9]

Another major component of fraction III displayed a correlation between a nitrogen signal at δ 375.0 and two protons at δ 7.51 and 2.81 (region IIIb, Fig. 1). In the $^1H,^1H$ COSY and ^1H–^{13}C HMBC spectra, the former proton signal was recognised as part of a set of three coupled resonances appearing at δ 7.51 (1H, d, J=12 Hz), 7.25 (1H, dd, J=15.2, 12.0 Hz) and 6.62 (1H, d, J=15.2 Hz) which showed one-bond correlations with carbons at δ 131.6, 133.6 and 138.2, in that order. The 7.51 proton signal was additionally split by scalar coupling with the ^{15}N nucleus (J=3.6 Hz). Moreover, the protons at δ 7.51 and 2.81 showed long range correlations with carbon signals at δ 138.2 (CH) and a 158.3 (quaternary carbon), whereas the protons at δ 7.25 and 6.62 showed cross peaks with carbon

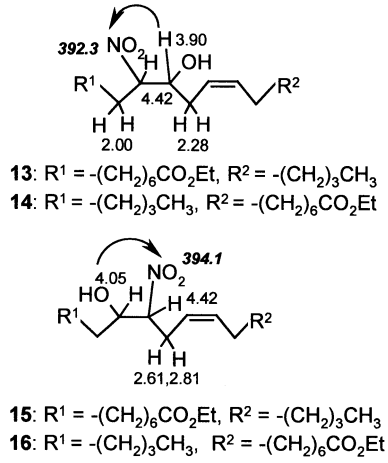
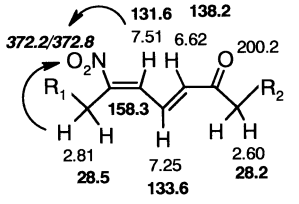


Figure 5. Structures and ^1H–^{15}N correlations of 1,2-nitrohydroxyalkene products in fraction III. Arrows indicate ^1H–^{15}N correlations.



17: R_1= -$(CH_2)_6CO_2Et$, R_2 = -$(CH_2)_3CH_3$
18: R_1= -$(CH_2)_3CH_3$, R_2 = -$(CH_2)_6CO_2Et$

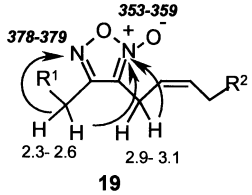
Figure 6. Structure and assignment of diagnostic proton, carbon (enbolded) and nitrogen (italicized) resonances of the yellow component of fraction III. Arrows highlight $^1H-^{15}N$ correlations.

resonances at δ 131.6 and 158.3 as well as with a carbonyl resonance at δ 200.2. Cross peaks with the latter resonance was also exhibited by a distinct triplet at δ 2.81 showing one bond correlation with a carbon at δ 28.5. Repeated TLC of fraction III eventually allowed isolation of a yellow product (λ_{max} 281, 383) with spectral features identical with those of the above species. GC–MS analysis indicated an intense peak at *m/z* 370 and a fragmentation peak at *m/z* 352 ($M-{}^{15}NO_2$). On this basis the product was assigned regioisomeric structures **17/18** featuring the 5-nitro-2,4-pentadienone moiety (Fig. 6).

The ^1H, ^{15}N HMBC spectrum showed also weak but well discernible cross peaks between a group of ^{15}N resonances at δ 353–359 and proton resonances at δ 3.1–2.9 and 2.3–2.6 (region IIIc, Fig. 1), and between the latter and nitrogen resonances at δ 378–379. These values were suggestive of 2-oxido-1,2,5-oxodiazole (furoxan) derivatives of the type **19** (Fig. 7).[11,12] Unfortunately this hypothesis remained unsubstantiated because of exceedingly overlapped proton resonances hindering analysis of the ^1H,^{13}C NMR correlation spectra.

2.5. Reaction of 1,4-hexadiene with acidic nitrite

To gain a deeper insight into the acid-promoted reaction of 1,4-pentadiene systems with nitrite ions, the study was extended to 1,4-hexadiene (80% *E* isomer) as a simple skipped diene. Exposure of the compound to nitrite (5 M equiv.) in 1% sulfuric acid resulted in a fast reaction leading to a relatively simple pattern of products with a main UV absorbing species positive to the Griess reagent. The compound was obtained by preparative TLC as a chromatographically homogeneous band. The ^1H NMR spectrum showed a mixture of stereoisomers (90% *E* and 10% *Z*) in which the less substituted alkene moiety was fully substituted. Moreover the bis-allylic methylene group was shifted



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

Figure 7. Proposed structural assignments for the minor, putative furoxan components of Fraction III. Arrows indicate $^1H-^{15}N$ correlations.

downfield by ca. 0.8 ppm, suggesting a deshielding group on the C-2 carbon. Scrutiny of the ^1H, ^{13}C HMBC spectrum revealed correlations between the methylene protons and four carbon resonances at δ 109.4, 120.3, 133.2 and 159.0. GC–MS analysis of the isolated band showed a main peak giving a molecular ion peak at *m/z* 185, consistent with a nitrofuroxan derivative, and a diagnostic fragmentation peak at *m/z* 139 due to loss of a nitro group. On this basis the compound was formulated as 3-(2-butenyl)-4-nitro-2-oxido-1,2,5 oxadiazole (**20**).

The remainder of the mixture was accounted for by the starting material and nitrated products which eluded detailed characterisation.

2.6. Mechanistic remarks

The structures of the main products formed by exposure of ethyl linoleate to nitrite ions in an acidic medium confirms the prevalence of nitration reactions. These would derive from homolytic attack of NO_2 to the skipped diene system of ethyl linoleate generating β-nitroalkyl radicals.[9] Free radical recombination with another molecule of NO_2 would then lead to 1,2-nitronitrite intermediates which may suffer HNO_2 elimination and/or hydrolysis to give nitroalkenes **1–4** and/or 1,2-nitroalcohols **13–16**, respectively. Alternatively, H-atom abstraction would afford 3-nitro-1,5-hexadiene derivatives (**5/6**) and 1,5-dinitro-1,3-pentadiene products (**9/10**).[9]

The identification of 1,2-nitronitrates (**11/12**), which account for at least 25% of the reacted material based on ^1H NMR analysis, is paradigmatic of the improved analytical potential of the integrated approach reported in this paper. These products would arise by oxidation of 1,2-nitronitrite intermediates. Another viable route would involve trapping of the initially formed β-nitroalkyl radical by oxygen followed by coupling with NO to give a nitrosoperoxyl intermediate which then rearranges to a nitrate.[13–15] However, the virtually identical product pattern formed in an oxygen-free atmosphere rules out the prevalence of oxygen-dependent routes under the specific reaction conditions of this study.

Formation of the *Z*-nitroalkenes **7/8** does not conform with the expected stereochemical course of alkene nitration, leading mainly to the *E* isomers.[4] It is plausible that in the case of ethyl linoleate the *Z*-nitroalkene route is less unfavourable because of the steric hindrance of the alkyl chains on the double bonds, weakening the role of the nitro group as the main determinant of the stereochemical outcome of the reaction.

The mechanism of formation of the 5-nitrodienones **17/18** is not straightforward. In principle, a carbonyl-forming route from hydroperoxide precursors, preceding or following the nitration step, would be a tenable hypothesis. However, in separate experiments it was found that neither (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoic acid nor (9*Z*,11*E*)-13-oxo-9,11-octadecadienoic acid, the corresponding ketone, when reacted with acidic nitrite under the same conditions as ethyl linoleate, were converted to significant amounts of **17/18** as free acids. Moreover,

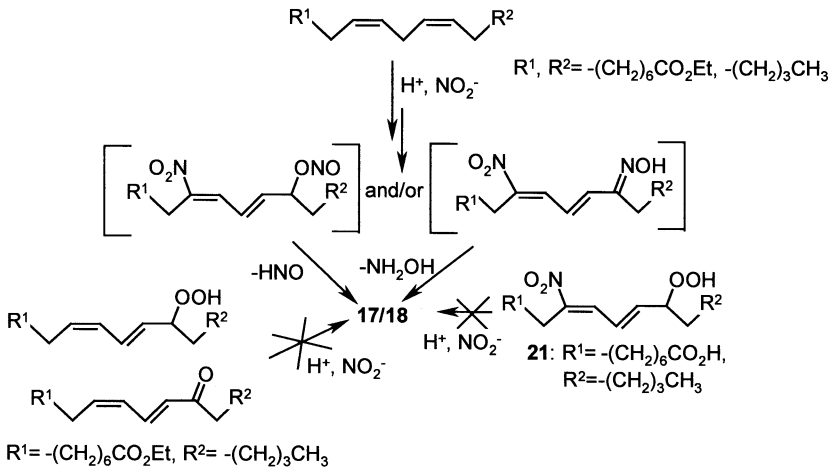

Figure 8. Proposed mechanism of formation of 5-nitrodienones **17/18**.

(9*E*,12*E*,13*S*)-13-hydroperoxy-9-nitro-9,11-octadecadienoic acid (**21**), prepared as recently reported,[16] failed to give detectable **17/18** free acids by exposure to acidic nitrite or atmospheric oxygen in the presence of iron ions for prolonged periods of time (up to 10 h), i.e. under conditions favouring conversion of hydroperoxides to ketones.[17] Considering that the reaction course is not substantially modified in an oxygen-free medium, and that formation of the yellow nitrodienone is not appreciable during the very early stages of the reaction, it can be suggested that this species originates by a complex pathway involving H-atom abstraction to afford eventually nitronitrite or nitro-nitroso (nitrooxime) intermediates susceptible to carbonyl-forming decomposition or hydrolysis (Fig. 8).

The different reactivity of 1,4-hexadiene, yielding nitro-furoxan **20** as main product, would reflect the presence of the singly substituted double bond, which is lacked by ethyl linoleate. Thus, nitration at this double bond and subsequent conversion of the resulting 1-nitro-1,4-hexadiene to a 1,2-dinitro-1-nitroso intermediate (or the 1,2-nitrooxime tautomer) would be a plausible route to compound **20** (Fig. 9).[12,18] The proclivity of nitrogen oxides to react at terminal sites of 1-alkenes is well documented,[19,20] and so is furoxan formation by reaction of polar double bonds bearing electron-withdrawing groups (e.g. cinnamic acids,[12,21] methacrylic acid,[12] cinnamaldehyde[12]) with acidic nitrite. Formation of the putative 1,2-nitronitroso (nitrooxime) precursors may proceed by a heterolytic pathway, a guess supported by the polar nature of the nitro-substituted double bond.

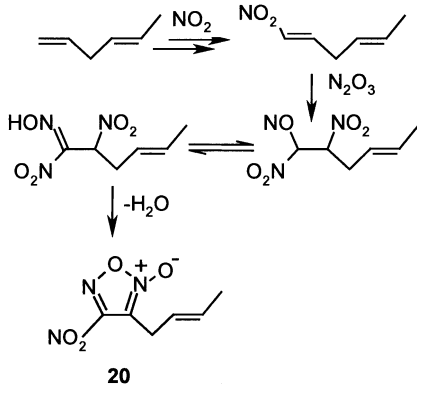

Figure 9. Proposed formation route of nitrofuroxan **20**.

3. Conclusions

The integrated NMR/GC–MS methodology described in this paper allowed an unprecedented insight into the acid-promoted reaction of an unsaturated fatty acid with nitrite ions. The ^{15}N tracer approach has the advantage that few or no processing steps are required, and ensures that all nitrogenous reaction products are selectively detected even in the presence of abundant non-nitrogenous species, minimising the risk that important nitrosation/nitration products were lost or overlooked. This methodology overcomes the typical limitations of GC–MS analysis due to the volatility and the intrinsic stability of the analytes, which is particularly unfavourable in the most polar components.

The inventory of nitration/nitrosation products reported in this paper and the rationalisation of their formation integrate and expand current knowledge of the reactivity of 1,4-dienes with nitrogen oxides derived from acidic nitrite. Since the skipped diene is the core structural feature of prominent groups of naturally occurring lipids, the observed reactivity toward nitrogen oxides may be of relevance to the bioactivity of these compounds.

Finally, the present work may provide the background to set future studies on nitrite-mediated nitration/nitrosation processes in complex matrices and biological systems.

4. Experimental

4.1. General

Ethyl linoleate (98%), 1,4-hexadiene (99%, *E*,*Z* mixture), [^{15}N]$NaNO_2$ (>99%) were purchased from Aldrich and used as obtained. GC–MS was carried out on a HP 5889A or 5970 instrument coupled with a quadrupole mass spectrometer using a 30 m Rtx-5MS-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. Data were processed using G1701AA data analysis software. The temperature program of the column was as follows: at 40°C, hold time=1 min; from 40 to 280°C, rate=5°C/min; hold at 260°C for 11 min. The injector and detector were taken at 220 and 250°C, respectively. The acquisition started 5 min after the injection (solvent delay 5 min), and was set in scan mode in the range 43–550 amu. The EI spectra were obtained at 70 eV. For CI spectra the source and quadrupole were taken at 200 and 100°C, respectively, and a mass range 40–600 amu was chosen.

UV and IR spectra were performed using a diode array and a FT-IR spectrophotometer, respectively. ^{1}H, ^{13}C, ^{15}N NMR spectra were recorded at 400.1, 100.6 and 40.5 MHz, in that order using a Bruker DRX-400 MHz instrument fitted with a 5 mm ^{1}H/broadband gradient probe with inverse geometry. Standard Bruker implementations of gradient-selected versions of inverse (^{1}H detected) heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) experiments were used. The HMBC experiments used a 100 ms long-range coupling delay. Samples analysed by NMR spectroscopy were dissolved in $CDCl_3$. Chemical shifts are recorded in δ values (ppm) downfield from tetramethylsilane (^{1}H and ^{13}C NMR) or with reference to [^{15}N]urea in DMSO at 76.97 ppm, relative to NH_3 (liquid, 298 K) at 0.0 ppm (^{15}N NMR).[22] Analytical and preparative TLC analyses were performed on F254 0.25 and 0.5 mm silica gel and high performance (HPTLC) plates from Merck. Griess reagent (1% sulphanylamide and 0.1% naphthylethylenediamine in 5% phosphoric acid), potassium dichromate in 20% sulphuric acid and iodine were used for product detection on TLC plates.

4.2. Reaction of linoleic acid ethyl ester with [^{15}N]$NaNO_2$

To linoleic acid ethyl ester (462 mg, 1.5 mmol) in 1% sulfuric acid (20 mL) [^{15}N]labelled or unlabelled sodium nitrite (420 mg, 6.0 mmol) was added while the mixture was taken under vigorous stirring in a stoppered round-bottom flask at room temperature. After 3 h, the mixture was extracted with cyclohexane (3×10 mL) and the combined organic layers washed with brine and dried over sodium sulfate to afford a yellow residue. Fractionation of the resulting residue on PTLC using cyclohexane/ethyl acetate 95:5 as the eluant afforded four main fractions. The R_f 0.7 fraction (200 mg) consisted of the starting material and was not further analysed. Fractions at R_f 0.45–0.60 (55 mg, fraction I), R_f 0.35–0.40 (32 mg, fraction II) and R_f 0.15–0.30 (40 mg, fraction III) were subjected to NMR and GC analysis.

4.3. Reaction of 1,4-hexadiene with nitrite

To 1,4-hexadiene (304 mg, 3.7 mmol) in 1% sulfuric acid (20 mL) sodium nitrite (1.28 g, 18.5 mmol) was added and the mixture was taken under vigorous stirring in a stoppered round-bottom flask at room temperature overnight. The mixture was extracted with ethyl acetate and the residue obtained (151 mg) was fractionated by preparative TLC using cyclohexane/ethyl acetate 7:3 to give a main band (25 mg, 4% yield) consisting of pure **20**. UV λ_{max} (cyclohexane) 308 nm; FT-IR ($CHCl_3$) ν_{max} 1635, 1571, 1499, 1354 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) 1.69 (2.4 H, d, J=7.6, 0.8 Hz), 1.76 (0.6 H, d, J=7.6, 0.8 Hz), 3.53 (1.6 H, dd, J=6.4 Hz), 3.62 (0.4 H, d, J=6.4 Hz) 5.41 (1H, m), 5.75 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) 18.6, 27.0, 109.4, 120.3, 133.2, 159.0; EI MS m/z 185 (M^+, 40), 139 ($M-NO_2$, 72), 97 ($M-NO_2-C_3H_6$, 100); HR MS calc for $C_6H_7N_3O_4$ 185.1404, found m/z 185.1415.

4.4. Isolation of 17/18 and formation from putative precursors

Fraction III (40 mg) obtained from the reaction mixture of ethyl linoleate with $NaNO_2$ was purified by preparative TLC (cyclohexane/ethyl acetate 90:10) to yield a yellow band (10 mg) which was subjected to further fractionation (cyclohexane/ethyl acetate 95:5) to afford **17/18** (5 mg). UV λ_{max} 281, 383 nm; ^{1}H NMR ($CDCl_3$) (see Fig. 6); NICI MS R_t 46.50 min, m/z 370.

(9*E*,11*E*)-13-Hydroperoxy-9-nitrooctadecadienoic acid (**21**) was prepared as previously described.[16] (9*Z*,11*E*, 13*S*)-13-hydroperoxyoctadeca-9,11-dienoic acid was obtained by lipoxidase oxidation as described[23] and purified by preparative TLC (cyclohexane/ethyl acetate 7:3). The isolated product was exposed to Fe(II)/H_2O_2 in 0.1 M phosphate buffer to give (9*Z*,11*E*)-13-oxo-9,11-octadecadienoic acid[17]as the main product which was isolated by preparative TLC (cyclohexane/ethyl acetate 7/:3). Purity of the compound was checked by NMR analysis: ^{1}H NMR ($CDCl_3$) δ (ppm) (selected resonances): 0.89 (3H, m) 1.29–1.63 (16H, m), 2.28 (4H, m), 2.56 (2H, t, J=7.2 Hz), 5.88 (1H, m), 6.09–6.18 (2H, m), 7.49 (1H, dd, J=14.2, 12 Hz).

Compound **21** or (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoic acid or (9*Z*,11*E*)-13-oxo-9,11-octadecadienoic acid were exposed separately to nitrite in 1% sulphuric acid as detailed above. Periodically, aliquots of the reaction mixture were extracted with ethyl acetate and analysed by TLC (cyclohexane/ethyl acetate 7:3) and ^{1}H NMR. In other experiments **21** (0.5 mM) was exposed to iron(II) ions (0.2 M equiv.) in 0.1 M phosphate buffer (pH 7.4). After 10 h, the reaction mixture was extracted with ethyl acetate and analysed as above.

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