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Oxygen-Dependent Nitration of Ethyl Linoleate with Nitric Oxide.

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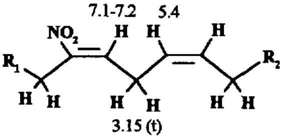
Abstract. Exposure of linoleic acid or its ethyl ester to NO in air-equilibrated cyclohexane led to an exceedingly complex pattern of products, a fraction of which (30-40% w/w) consisted of an unseparable mixture of isomeric nitroolefin and nitronitrate derivatives, including **1-3**.

Increasing experimental evidence implicates nitric oxide (NO)[1] as a critical modulator of lipid peroxidation processes and tissue injury during inflammation, in cerebral ischemia and in oxidative stress-dependent neurodegenerative disorders[2,3]. The detailed mechanisms and outcome of these interactions remain however elusive, because of the inherent complexity of the chemistry involved, and most of the present knowledge rests on the loose identification by MS techniques of nitrosoperoxy- and hydroperoxynitrosoperoxy- adducts formed by peroxidation of linolenic acid in the presence of NO[3].

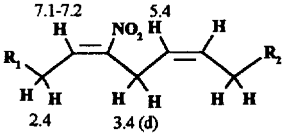
In pursuing a programme on the mechanisms of NO-induced neurotoxicity[4,5], we were recently prompted to approach this issue by investigating the reaction of NO with linoleic acid as a model unsaturated lipid. When purified NO was slowly bubbled for 5-10 min through a vigorously stirred solution of the acid or its ethyl ester (1×10^{-3} M) in air-equilibrated cyclohexane, a fast reaction occurred leading to an exceedingly complex pattern of products, most of which positive to the Griess reagent for nitrite-releasing groups. No product formation was observed when air was rigorously excluded from the medium. In the case of ethyl linoleate, TLC fractionation of the mixture afforded a chromatographically homogeneous colourless oil (about 30-40% yield w/w; $R_f = 0.75$ in cyclohexane/ethyl acetate 7:3 v/v; UV: shoulder at 240 nm), which proved to consist of an intimate mixture of products resistant to all attempts at separation.

GC-MS analysis revealed four poorly resolved peaks giving fragmentation patterns at m/z 336, 308, 277, 196, 150 (peak 1); 336, 296, 250 (peak 2); 336, 308, 277, 196, 150 (peak 3); 336, 296, 250 (peak 4). The same fragmentation peaks could be discerned in the EI-MS spectrum, which featured also weak or barely detectable peaks at m/z 387, 371, 355 and 324. The FT-IR spectrum ($CHCl_3$) displayed strong bands at 1724, 1646, 1558, 1520, 1375, 1337, 1278 and 848 cm^{-1}, suggesting the presence of nitro groups linked to sp^2 and sp^3 carbons and, apparently, a nitrate ester functionality. Scrutiny of the ^{1}H- and ^{13}C-NMR spectra revealed complex and largely overlapped patterns of resonances which, however, could be substantially resolved with the aid of DEPT and 2-D correlation experiments. Particularly revealing was a multiplet at δ 7.10-7.20 (about 0.45 H, relative area determined with respect to the O-CH_2 quartet) correlating with CH carbons at δ 137, which pointed, together with quaternary carbons at δ 147, 151 and 153, to (E)-nitroalkene moieties. Other characteristic features included four sets of overlapped multiplets at δ 5.5-5.8, 5.5-5.7, 5.3-5.5 (about 2.4 H) and 4.9-5.0 (about 0.4 H), which correlated with four clusters of CH carbon resonances (from 6 to 12 discernible signals each) at δ 82-84, 133-137, 122-126 and 87-89, in that order. A doublet at δ 3.4 (about 0.6 H, J= 7 Hz), coupled to protons at δ 5.4; a triplet at δ 3.15 (0.3 H, J= 6.5 Hz), coupled to protons at δ 5.4 and 7.2; and a multiplet at δ 2.8 (about 1.1 H), coupled to protons at δ 5.4 and 4.9, could be assigned to a set of CH_2 groups resonating in the carbon spectrum in the range between δ 28 and 34. Both the chemical shifts and coupling patterns of these methylene groups were consistent with a common origin from the bis-allylic C-11 methylene of linoleate. An additional CH_2 signal (δ 2.4, m, δ 28) was coupled to protons at δ 7.10-7.20.

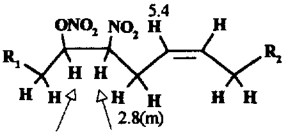
Overall, these data were strongly suggestive of a mixture of isomeric nitroalkene (**1** and **2**, about 45% of the fraction) and nitronitrate (e.g. **3**, about 40%) derivatives, arising probably *via* the initial addition of nitrogen dioxide, produced by autoxidation of NO, to the double bonds of ethyl linoleate[6]. Apparently, the remainder of the fraction consists of isomeric nitrocompounds related to **1-3**. Proposed structures for the chief components of the fraction with main proton resonance assignments are shown below.



1



2



$R_1,R_2=C_4H_9$ or $C_9H_{17}O_2$

3

In summary, the results of this study yield the first direct insight into the oxygen-dependent reaction of NO with linoleic acid, expanding available records of nitrations of alkenes with nitrogen oxides[7-10] to include 1,4-dienes, and providing an improved basis to inquire into the mechanisms of NO-induced cell damage.

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