The Chemistry of Peroxynitrite: Involvement of an ET Process in the Radical Nitration of Unsaturated and Aromatic Systems

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Keywords: Peroxynitrite / Nitration / Alkenes / Electron transfer / Nitrous anhydride

Reactions of peroxynitrous acid, HPN, with styrene under acidic conditions lead to the oxime **1**, the nitrate **2**, benzaldehyde (**3**), and α-nitroacetophenone (**4**) in overall yields that depend strongly on the pH value and with a product distribution that depends on the dioxygen concentration. The results are rationalized by assuming that HPN undergoes acid-catalyzed decomposition to give nitrous anhydride, or its synthetic equivalent, which is responsible for the regioselective nitration of the styrene double bond by an ET process. The resulting β-nitrobenzyl radical **6** can, depending on the reaction conditions, undergo reversible coupling with nitric oxide to afford the nitroso derivative **7** and then the tautomeric oxime **1**, or trapping by dioxygen, eventually leading to products **2**, **3**, and **4** through the intermediacy of the peroxynitrite derivative **8**. Oxime **1** and nitrate **2** are also obtained by treating styrene with nitrous anhydride under protic conditions, the latter being produced in situ from nitric oxide/dioxygen. Similarly to styrene, 1,4-diphenylbutadiene (**14**) gives radicals **22** and **21** by competitive trapping at the side chain and at the aromatic ring. In turn, radicals **22** and **21** undergo β-fragmentation reactions or trapping by dioxygen with eventual formation of nitrates **16** and **17**, cinnamic aldehyde (**18**), and the diol **15**. Finally, the HPN-promoted reaction of *p*-cresol (**27**) leads to the 2-nitro derivative **28** through an initial electron-transfer process followed by *in cage* recombination of the resulting radical ion pair.

Introduction

Peroxynitrite[2] has recently attracted the attention of chemists and biochemists since this species appears to be involved in the in vivo damage of several biological substrates.[3] The active oxidant in these processes has been generally believed to be the unstable peroxynitrous acid HPN (O=N−OOH) ($pK_a = 6.8$; $\tau_{1/2} < 1$ s),[4] which is generated from the relatively stable peroxynitrite anion PN ($O=N-OO^-$) at physiological pH values or lower. In vivo, the latter is formed from the reaction of nitric oxide (NO) with superoxide ($O_2^{\cdot -}$).[3a]

It has been hypothesized that HPN rapidly undergoes O−O bond scission with formation of an "in cage" radical pair, which can either act as a source of hydroxyl radicals and NO_2 (ca. 30%) or rearrange to nitric acid (70%).[5] However, it has been reported that HPN can also oxidize suitable substrates either by a direct mono- or di-electron transfer mechanism or by an indirect mono-electron mechanism.[4,6] Among biomolecules, sulfhydryl group containing proteins[7] and lipids containing polyunsaturated fatty acids[8] are key targets, although the actual mechanism of peroxynitrite reaction with both the sulfhydryl function and the carbon−carbon double bond is far from being fully understood.

In order to obtain a better understanding of these processes, we reasoned that preliminarily studies on the peroxynitrite-mediated reaction of simple molecules containing either the sulfhydryl group or the alkene double bond were required.

To this end, we have already studied the peroxynitrite-promoted reaction of simple thiols. In a recent paper, we reported[9] that PN is capable of oxidizing thiolate ions to sulfanyl radicals (and then to disulfide) under very basic conditions, whereas at acidic pH values HPN generates a nitrosating species, XNO, which leads to the formation of *S*-nitrosothiol derivatives as the main reaction products. Thiol nitrosation is strongly dependent on the pH value, indicating that HPN generates the nitrosating species XNO by an acid-catalyzed reaction.

In continuation of our studies, we report herein on the results obtained concerning the peroxynitrite-promoted reaction of simple alkenes carried out under conditions of both acidic and basic pH values, where the undissociated HPN and the dissociated PN forms, respectively, largely predominate.

Results and Discussion

Reactions of peroxynitrite under basic conditions were attempted by adding two molar equivalents of a 0.50 M aqueous basic solution (pH = 13.5) of peroxynitrite to a 0.10 M acetonitrile solution of the appropriate alkene (styrene or allylbenzene). The resulting mixtures were stirred at 10 °C for 30 min, then worked up as described in the Exp. Sect. In all cases, subsequent ^{1}H NMR analysis of the crude residue showed the exclusive presence of the unchanged alkene. No products derived from attack at the carbon−carbon double bond or, in the case of allylbenzene, at the allylic position, were detected.

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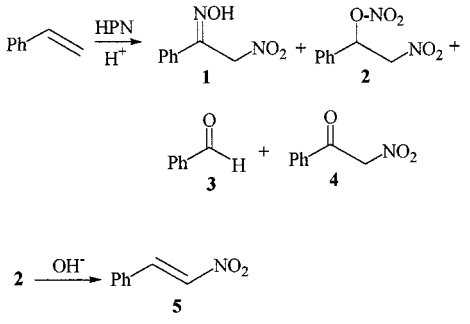
The above reactions were then repeated under acidic conditions under nitrogen by adding the basic solution of peroxynitrite to a solution of the alkene in a 10:3 acetonitrile/1.2 M hydrochloric acid mixture (final pH = 0). The resulting reaction mixtures were stirred for 1 h and then worked up as described in the Exp. Sect. Even under these acidic conditions, allylbenzene was found not to react at all. In contrast, styrene reacted to give α-nitroacetophenone oxime (**1**), 2-nitro-1-phenylethyl nitrate (**2**), benzaldehyde (**3**), and α-nitroacetophenone (**4**), together with an unidentified product that showed a singlet at δ = 5.35 in its ^{1}H NMR spectrum. These products were formed in a ratio of 74:17:1:5:4 and in 57% overall yield based on the amount of styrene used, as determined by ^{1}H NMR analysis (Table 1, Entry 1). Subsequent silica gel column chromatography allowed the separation of compounds **1**, **2**, and **4**. Oxime **1** was characterized by comparison of its spectral data with those reported in the literature,[10] whereas nitroacetophenone (**4**) was identified by comparison with an authentic commercial sample. Product **2** exhibited a ^{1}H NMR spectrum identical to that reported in the literature[11] for the regioisomeric 2-nitro-2-phenylethyl nitrate; the actual structural assignment was based on its ^{13}C NMR and mass spectra and on the finding that hydrolysis under basic conditions afforded β-nitrostyrene (**5**) in quantitative yield (Scheme 1). We were unable to isolate the unknown product by column chromatography and hence it remained uncharacterized. Benzaldehyde, formed in small amounts, was only identified by GC/MS and ^{1}H NMR analyses of the reaction mixture.

Table 1. Relative yields (%) of products **1**, **2**, **3**, and **4** obtained from reactions of styrene with peroxynitrite carried out at pH = 0 under nitrogen (A), air (B), and oxygen (C)

Entry	Conditions	Overall yield (%)[a]	**1**	**2**	**3**	**4**	Unknown product
1	A	57	74	17	1	5	4
2	B	40	18	62	3	17	trace
3	C	41	6	63	5	27	–

[a] Determined by ^{1}H NMR with acetophenone as an internal standard.



Scheme 1

To determine the effect of the amount of oxygen present in the medium, the reaction of styrene with peroxynitrite at pH = 0 was carried out under both air and oxygen. Under these conditions, compounds **1**, **2**, **3**, and **4**, as well as the unknown, were again found to be the exclusive reaction products, although in slightly lower overall yields (Table 1, Entries 2 and 3). More significantly, we found that the relative yields of benzaldehyde (**3**), nitroacetophenone (**4**) and, particularly, nitrate **2** strongly increased at the expense of the oxime **1**, as shown in Table 1.

The peroxynitrite-promoted reaction of styrene was repeated under nitrogen at various pH values in the range between 0.0 and 5.4. As indicated by the data presented in Table 2, the relative product yields were found to be independent of the pH value. In contrast, the overall yield sharply decreased on increasing the pH value, varying from 57% at pH = 0 (Table 2, Entry 1) to 13% at pH = 5.4 (Table 2, Entry 5).

Table 2. Relative yields (%) of products **1**, **2**, **3**, and **4** obtained from reactions of styrene with peroxynitrite carried out under nitrogen at different pH values

Entry	pH	Overall yield (%)[a]	**1**	**2**	**3**	**4**	Unknown product
1	0	57	74	17	1	5	4
2	1	33	75	12	2	6	4
3	1.4	29	77	15	2.5	7	5
4	1.7	28	75	11	2.5	4	5
5	5.4	13	78	2	2	7	12

[a] Determined by ^{1}H NMR with acetophenone as an internal standard.

The main results obtained from the peroxynitrite-promoted reaction of styrene can be summarized as follows: Carrying out the reaction under acidic conditions gave products **1**, **2**, **3**, and **4**, whereas no products were obtained under basic conditions. This indicates that the undissociated form HPN, and not the peroxynitrite anion PN, is involved as the reactive species.

The relative product yields were found to be strongly dependent on the oxygen concentration; under oxygen, the nitrate **2**, as well as nitroacetophenone (**4**) and benzaldehyde (**3**), were formed at the expense of the oxime **1**, which was the main product under nitrogen (Table 1). This finding led us to suggest the formation of the benzyl radical **6** as a key intermediate. In turn, radical **6** can be envisaged as formally deriving from regioselective radical nitration of the styrene double bond, i.e. through HPN behaving as a nitrating agent towards this double bond (Scheme 3).

However, independent experiments, in which styrene was treated with peroxynitrite at pH = 0 under nitrogen, showed that the overall yield of reaction products **1**, **2**, **3**, and **4** increased with the reaction time up to 30 min. Since HPN is very unstable (a lifetime of < 1 s has been reported),[4] we can easily infer that the actual species responsible for the formation of radical **6** is derived from HPN, but cannot be HPN itself.

Moreover, results obtained by treating styrene at different pH values (Table 2) showed that the overall yield of products **1**, **2**, **3**, and **4** strongly decreased on increasing the pH value, notwithstanding that over the entire range examined (0.0–5.4) HPN largely predominates over the undissociated form PN. On these bases, we suggest that the actual nitrating reagent is formed from HPN by an acid-catalyzed reac-

tion, probably involving the intermediacy of its protonated form, H_2PN^+ (Scheme 1).

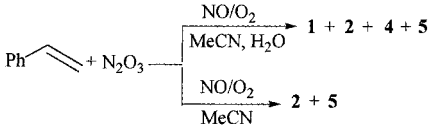
The present results parallel those we obtained regarding the reaction of HPN with thiols,[9] which led to *S*-nitrosothiols by nitrosation of the thiol group. The *S*-nitrosothiol yields, which increased with the reaction time up to 20 min, were found to be strongly dependent on the pH value. In this case, the involvement of the protonated form, H_2PN^+, was also invoked in the formation of the nitrosating species.

Our overall findings lead us to infer that acid-catalyzed decomposition of HPN leads to a reactive species, X−ONO, capable of behaving both as a nitrosating agent for thiols and as a nitrating agent for the styrene double bond. We have obtained evidence that this X−ONO species is a synthetic equivalent of nitrous anhydride, ON−ONO, or the anhydride itself.[12] In fact, by treating styrene with nitrous anhydride under various conditions, the latter being formed in situ from nitric oxide/dioxygen,[13] we obtained results that are essentially comparable with those obtained from the HPN-promoted reaction. When these reactions were carried out in acetonitrile/water solutions at both pH = 7 (Table 3, Entry 4) and pH = 0 (Table 3, Entries 1 and 3), we found that styrene was almost totally consumed to give the oxime **1** and the nitrate **2** as the main products, together with β-nitrostyrene (**5**) and small amounts of nitroacetophenone (**4**) (Scheme 2).

Table 3. Relative yields (%) of products **1**, **2**, **3**, **4**, and **5** obtained from reactions of styrene with nitric oxide carried out under air

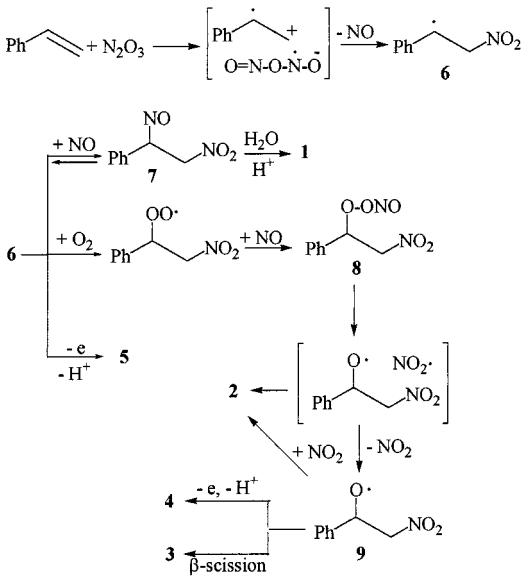
Entry		Overall yield (%)[a]	1	2	3	4	5	Unknown product
1	A[b]	85	64	14	trace	6	11	5
2	B[b]	87	–	45	trace	–	55	–
3	C[b]	80	70	17	trace	trace	9	4
4	D[b]	82	37	39	trace	3	19	2

[a] Determined by ^{1}H NMR with acetophenone as an internal standard. – [b] A: in acetonitrile acidified with 0.30 mL of 37% hydrochloric acid; B: in neat acetonitrile; C: in acetonitrile/1 M hydrochloric acid (5:2); D: in acetonitrile/water (5:2).



Scheme 2

On this basis, the formation of products **1** and **2** can be readily explained as outlined in Scheme 3. Reaction of nitrous anhydride, or its synthetic equivalent, with styrene could lead to the radical **6** and nitric oxide, from which the nitroso derivative **7** and, by subsequent tautomerization, the oxime **1** could be derived. In competition with the coupling with nitric oxide, the radical **6** could be trapped by dioxygen. Coupling of the resulting benzylperoxyl radical with nitric oxide would then give the peroxynitrite derivative **8**,[14] from which the nitrate **2** could be formed by O−O bond scission with formation of a benzyloxy radical **9**/nitric dioxide radical pair, followed by *in cage* recombination.[14]



Scheme 3

Alternatively, the benzyloxy free radical **9** can give rise to either benzaldehyde (**3**) by a β-scission process, to nitroacetophenone (**4**) by oxidation, or to the nitrate **2** by *out of cage* trapping by nitric dioxide (Scheme 3). In the reaction promoted by nitric oxide/dioxygen, the latter process largely predominates over the formation of **3** and **4** owing to the high nitric dioxide concentration produced under these conditions (Scheme 3).

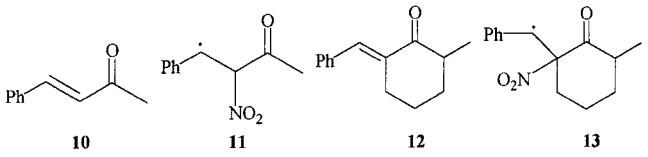
As mentioned above, the reaction of styrene with nitric oxide/dioxygen generated noticeable amounts of β-nitrostyrene (**5**), which was most likely derived from benzyl radical **6** by an oxidation/deprotonation process (Scheme 3). At the present time, we are unable to account for the fact that this product was not formed in the HPN-promoted reaction.

It is worth noting that no oxime **1** was formed when the reaction of styrene with nitric oxide/dioxygen was carried out in acetonitrile in the absence of water; in this case, the nitrate **2** and nitrostyrene (**5**) were the only reaction products (Scheme 2 and Table 3, Entry 2). According to the proposed mechanism, the absence of the oxime **1** under these latter conditions can be rationalized by assuming that benzyl radical **6** is trapped by nitric oxide in a reversible manner and that the acid-catalyzed tautomerization of the resulting nitroso derivative **7** to oxime **1** can only occur in a protic medium (Scheme 3). In the absence of water (or acid), only products deriving from the irreversible trapping of radical **6** by dioxygen can be formed.

At this stage a question arises, i.e. how nitrous anhydride can behave as a radical nitrating agent towards the styrene double bond. The finding that allylbenzene ($Ph-CH_2-CH=CH_2$), in contrast to styrene, does not react with peroxynitrite under acidic conditions[15] indicates a strong effect of the styrene phenyl ring in promoting the radical nitration of the alkene double bond.

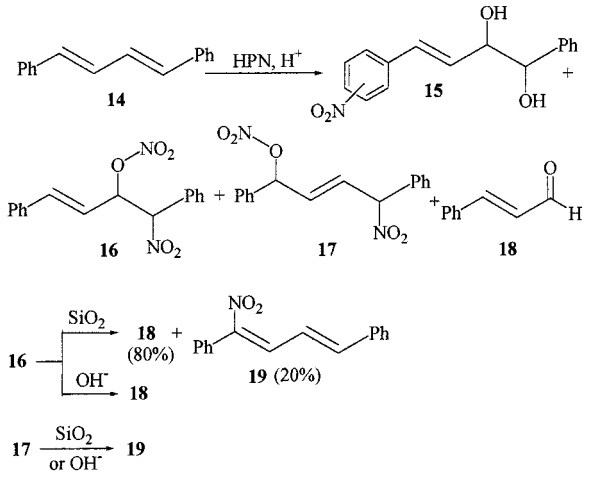
In principle, this effect could be explained by assuming that a direct radical addition of nitric dioxide (present in equilibrium with nitrous anhydride) occurs, leading to the resonance-stabilized benzyl radical **6**. However, we have obtained evidence that an electron-transfer (ET) process between styrene and nitrous anhydride (or its synthetic equivalent) is probably involved rather than a direct radical nitration. From such an ET process, a radical ion pair would initially be formed, from which radical **6** could arise from *in cage* coupling with nitric oxide loss (Scheme 3). Supporting evidence in favor of the ET mechanism was provided by the results of peroxynitrite-promoted reactions of benzalacetone (**10**), 2-methyl-6-[(*E*)-phenylmethylidene]cyclohexanone (**12**), and 1,4-diphenylbutadiene (**14**) carried out under acidic conditions.

In fact, benzalacetone (**10**) and 2-methyl-6-[(*E*)-phenylmethylidene]cyclohexanone (**12**) did not react at all with peroxynitrite under either oxygen or nitrogen, notwithstanding the presence of the styrene phenyl ring capable of stabilizing the possible benzyl-type radical intermediates **11** and **13**. In terms of the ET hypothesis, the lack of reactivity of these styrene derivatives **10** and **12** can probably be attributed to their higher ionization potentials[16] due to the conjugated electron-withdrawing carbonyl groups.



10 **11** **12** **13**

In contrast, 1,4-diphenylbutadiene (**14**) was quantitatively converted under oxygen to give the diol **15** (15%), the 1,2-adduct **16** (10%), the 1,4-adduct **17** (65%), and cinnamic aldehyde (**18**) (10%) (Scheme 4). The aforementioned yields were determined by ^{1}H NMR analysis of the crude mixture. Besides these products, subsequent column chromatography also resulted in the separation of the nitrodiene **19**.



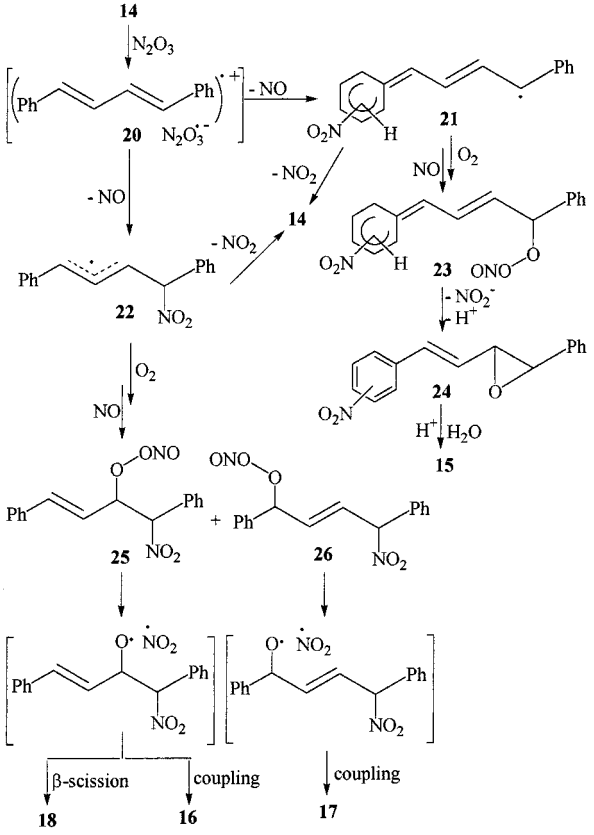
Scheme 4

Independent experiments showed that **19** was formed from both nitrate derivatives **16** and **17** as a result of silica gel catalyzed decomposition. In fact, after absorption onto silica gel for 24 h, the nitrate **17** was quantitatively converted into nitrodiene **19**, whereas the nitrate **16** was converted into an 80:20 mixture of cinnamic aldehyde (**18**) and nitrodiene **19**. On the other hand, both **16** and **17** were found to be essentially stable under the reaction conditions, whereas they undergo slow decomposition on standing in air or rapid hydrolysis under basic conditions. Upon treatment with 10% aqueous NaOH, **17** was quantitatively converted into diene **19**, and **16** into cinnamic aldehyde (**18**) (Scheme 4).

The hitherto unknown products **15**, **16**, and **17** were characterized on the basis of their IR, NMR, and MS data. In particular, ^{1}H NMR analysis showed that all three compounds were formed as diastereomeric mixtures in the (*E*) configuration. Positive-ion electron-impact mass spectrometry was not useful for these compounds, since molecular ions were not detected. In contrast, all of them showed the molecular ion peaks in their negative-ion electron-impact mass spectra. Nitrates **16** and **17** showed, in addition to the negative molecular ion at m/z = 314, key fragment ions at m/z = 252 [$M^{\cdot} - NO_3^{\cdot}$], 147 [$Ph-C{=}C-NO_2^{\cdot -}$], and 46 [NO_2^-]. The diol **15** showed the negative molecular ion at m/z = 285 and notable fragment ions at m/z = 178 [$M^{\cdot -} - PhCHOH^{\cdot}$], 123 [$PhNO_2^{\cdot -}$], and 46 [$NO_2^-$]. Both **16** and **17** showed a very strong band at 1555 cm^{-1} in their IR spectra. Elemental analysis was performed for compound **15**, but not for **16** and **17** owing to the impossibility of obtaining pure samples. However, compound **15** has not quite been fully characterized as it has not been possible to ascertain the *ortho* or *para* position of the nitro group by ^{1}H NMR analysis.

According to the reaction mechanism suggested for styrene, the formation of products **15**–**18** can most probably be explained by assuming that 1,4-diphenylbutadiene, owing to its low ionization potential,[16] undergoes a facile oxidation to the corresponding radical cation **20** (Scheme 5). This latter can be trapped by the $N_2O_3^{\cdot -}$ counterion, either at the allylic position, leading to radical **22**, or at the aromatic ring, leading to radical **21**. Radical **22** can be trapped by dioxygen at either the 2- or 4-position, ultimately leading to peroxynitrite derivatives **25** and **26**. In analogy to the behavior exhibited by **8** (see Scheme 3), **25** and **26** can give nitrates **16** and **17**, respectively, through an O–O bond scission/recombination process. Furthermore, peroxynitrite derivative **25** can give cinnamic aldehyde (**18**) through an O–O bond scission/β-fragmentation process. In turn, radical **21** can be trapped by dioxygen to eventually afford the peroxynitrite derivative **23**. This latter might lead to oxirane **24** through attack of the electrophilic peroxidic oxygen atom on the adjacent carbon–carbon double bond with concomitant loss of NO_2^-. The driving force behind this reaction can be envisaged as being the subsequent aromatization by proton loss. From **24**, the diol **15** can be smoothly formed by hydrolysis under the reaction conditions (Scheme 5).

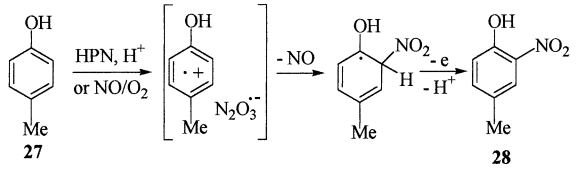
It is noteworthy that unchanged 1,4-diphenylbutadiene (**14**) was recovered in 90% yield when the reaction was car-



Scheme 5

ried out in the absence of oxygen (under argon or nitrogen). Only small amounts of compounds **15**−**18** and some unidentifiable products were detected by ^{1}H NMR. This finding suggests that radicals **21** and **22** undergo, in competition with trapping by dioxygen, β-fragmentation with formation of the starting diene **14** rather than trapping by nitric oxide, as in the case of styrene (Scheme 5).

Trapping of radical cation **20** at the aromatic ring to give radical **21** is consistent with our results obtained from a brief investigation of the HPN-promoted reaction of aromatic substrates, i.e. acetophenone and *p*-cresol (**27**). As expected, acetophenone, due to its high ionization potential,[16] did not react at all under any conditions. In contrast, *p*-cresol (**27**), a highly oxidizable aromatic substrate,[16] reacted with peroxynitrite to give 4-methyl-2-nitrophenol (**28**) in a manner strongly dependent on the pH value (90% yield at pH = 0; 4% at pH = 7). The formation of **28** can easily be rationalized in terms of initial oxidation of *p*-cresol to the corresponding radical cation followed by *in cage* recombination with the radical counterion, $N_2O_3^{\bullet-}$. The nitrophenol **28** was also obtained in high yields (> 90%) by treating *p*-cresol with nitric oxide/dioxygen in acetonitrile/water solution, both under acidic and neutral conditions (Scheme 6).



Scheme 6

Conclusions

Our present and previous results, even though referring to reactions carried out under conditions very different to physiological ones, allow some conclusions to be drawn concerning the interaction mechanism of peroxynitrite with biological targets such as the thiol function and the alkene double bond. It appears that dissociated PN can only behave as a mild oxidant, capable of oxidizing the thiolate group to sulfanyl radicals, but is incapable of oxidizing the styrene double bond. On the other hand, under acidic conditions the undissociated form, HPN, behaves as a source of nitrous anhydride, or its synthetic equivalent, which reacts as either a nitrosating or nitrating agent, depending on the nature of the substrate.

It has previously been reported that thiols are readily converted into the corresponding *S*-nitrosothiols.[9] In the present work, we show that the nitration of substrates having suitably low ionization potentials can occur at both the alkene double bond and the benzene ring. The reaction involves initial electron transfer followed by coupling of the resulting radical ions with loss of nitric oxide.

Reaction of styrene leads to the benzyl radical **6**, which, depending on the reaction conditions, can afford the nitroso derivative **7** and, by reversible coupling with nitric oxide, the tautomeric oxime **1**, or undergo competitive trapping by dioxygen, eventually leading to products **2**, **3**, and **4** through the intermediacy of the peroxynitrite derivative **8**. The overall yield of the reaction products decreases with increasing pH value, whereas the relative yield ratio **1**/(**2** + **3** + **4**) is strongly dependent on the dioxygen concentration.

Similarly to styrene, 1,4-diphenylbutadiene (**14**) gives nitro radicals **22** and **21** by competing coupling at the side chain and at the aromatic ring. In turn, radicals **22** and **21** undergo β-fragmentation reactions or trapping by dioxygen with the eventual formation of nitrates **16** and **17**, cinnamic aldehyde (**18**), and the diol **15**.

Finally, the peroxynitrite-promoted reaction of *p*-cresol (**27**) leads to the 2-nitro derivative **28** by an initial electron-transfer process followed by *in cage* recombination of the resulting radical ion pair.

Experimental Section

General: NMR spectra were recorded with a Varian Gemini 200 (or 300) instrument using Me_4Si as an internal standard. − GC-MS analyses were performed with a Carlo Erba QMD 1000 instrument. − Mass spectra were recorded with a VG 7070E instrument operating in electron-impact ionization mode. − UV/Vis spectra were recorded with a Perkin−Elmer Lambda 12 instrument. − IR spectra were recorded from samples in $CHCl_3$ solution with a Perkin−Elmer FT-IR 1600 instrument.

Materials: Styrene, benzalacetone (**10**), *p*-cresol (**27**), acetophenone, and allylbenzene were purchased from commercial sources. 2-

Methyl-6-[(*E*)-phenylmethylidene]cyclohexanone (**12**)[17] and 1,4-diphenylbutadiene (**14**)[18] were prepared as described in the literature. Peroxynitrite was synthesized according to the previously reported procedure;[9] its concentration (usually in the range 0.45−0.50 M) was determined spectrophotometrically (ε_{302} = 1670 $M^{-1}cm^{-1}$).[19] Solutions kept at −18 °C showed little decomposition over several weeks.

Reactions of Peroxynitrite under Basic Conditions. − General Procedure: To a 0.10 M acetonitrile solution of the appropriate alkene (styrene or allylbenzene) (10 mL), a 0.50 M aqueous solution of peroxynitrite (4 mL) (pH = 13.5) was added with stirring at 5−10 °C. The resulting solution was stirred for 1 h, then extracted with diethyl ether. The organic layer was washed twice with water and then the solvent was removed. In all cases, ^{1}H NMR analysis of the recovered residue showed the exclusive presence of the respective unchanged alkene.

Reactions of Peroxynitrite under Acidic Conditions. − General Procedure: To a 0.10 M acetonitrile solution (20.0 mL) of the appropriate substrate {styrene, allylbenzene, benzalacetone (**10**), 2-methyl-6-[(*E*)-phenylmethylidene]cyclohexanone (**12**), acetophenone, 1,4-diphenylbutadiene (**14**), or *p*-cresol (**27**)} (2.0 mmol), the requisite amount of 1.2 M hydrochloric acid (3.0−6.0 mL) was added at 5−10 °C and then the appropriate gas (air, dioxygen, or nitrogen) was bubbled through the resulting solution. After 20 min, a 0.50 M aqueous solution of peroxynitrite (8.0 mL) was added with stirring. The reaction mixture was stirred for 1 h and then diethyl ether (30 mL) was added. The aqueous phase was separated and the pH value was measured (see Table 2). The organic layer was washed twice with water and the solvent was evaporated.

Reactions of Allylbenzene, Benzalacetone (10), 2-Methyl-6-[(*E*)-phenylmethylidene]cyclohexanone (12), and Acetophenone: These reactions were performed following the addition of 6.0 mL of 1.2 M hydrochloric acid (final pH = 0). ^{1}H NMR analyses of the resulting reaction mixtures showed the exclusive presence of the respective unchanged starting materials.

Reaction of Styrene: The mixture obtained from the reaction carried out under nitrogen at pH = 0 (6.0 mL of 1.2 M hydrochloric acid added) was chromatographed on a silica gel column. Gradual elution with petroleum ether (b.p. 40−60 °C)/diethyl ether afforded unchanged styrene, oxime **1**[10] (125 mg, 35%), nitroacetophenone (**4**) (10 mg, 3%), and the nitrate **2**[11] (40 mg, 9%). Products **1** and **4** were identified by comparison of spectral data with those reported in the literature[10] and by comparison with an authentic specimen, respectively. The nitrate **2** {^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.58 (dd, 1 H, J_1 = 14.8 Hz, J_2 = 3.7 Hz), 4.80 (dd, 1 H, J_1 = 14.8 Hz, J_2 = 10.2 Hz), 6.56 (dd, 1 H, J_1 = 10.2 Hz, J_2 = 3.7 Hz), 7.35−7.60 (m, 5 H). − ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = 76.2 (CH_2), 80.3 (CH), 127.4 (CH), 130.2 (CH), 131.1 (CH), 139.6 (C). − MS (EI): *m*/*z* (%) = 149 [M^+ − 63] (20), 120 (40), 105 (100), 91 (40), 77 (50), 52 (60)} showed ^{1}H NMR signals identical to those reported in the literature[11] for the regioisomeric 2-nitro-2-phenylethyl nitrate. Its structural assignment was based on chemical evidence: treatment with 1.0 M aq. sodium hydroxide solution at room temperature for 12 h quantitatively afforded β-nitrostyrene, which proved to be identical to an authentic specimen. GC-MS and ^{1}H NMR analyses of the reaction mixture showed, in addition to products **1**, **2**, and **4**, the presence of a small amount of benzaldehyde (**3**). The reaction at pH = 0 (6.0 mL of 1.2 M hydrochloric acid added) was also carried out under air, oxygen, and nitrogen. The resulting reaction mixtures were quantitatively analyzed by ^{1}H NMR by considering signals at δ = 5.62 for oxime **1**, δ = 4.58 for nitrate **2**, δ = 9.98 for benzaldehyde (**3**), and δ = 5.91 for nitroacetophenone (**4**), using acetophenone (0.30 mmol) as an internal standard. The relative yields thus determined and the overall yields, which are referred to the starting styrene, are reported in Table 1. The reaction under nitrogen was also carried out at various pH values (1.0, 1.4, 1.7, 5.4). The reaction mixtures obtained were analyzed as described above. The relative yields thus determined and the overall yield, which is referred to the starting styrene, are reported in Table 2.

Reaction of 1,4-Diphenylbutadiene (14): The reaction was carried out at pH = 0 (3.0 mL of 1.2 M hydrochloric acid added). ^{1}H NMR analysis of the mixture obtained from a reaction carried out under oxygen showed the absence of starting diene and the presence of the 1,4-adduct **17** (65%), the 1,2-adduct **16** (10%), the diol **15** (15%), and cinnamic aldehyde (**18**) (10%). Subsequent silica gel column chromatography gave, on gradual elution with petroleum ether (b.p. 40−60 °C)/diethyl ether, the following products: (***i***) 1-Nitro-1,4-diphenylbutadiene (**19**) (110 mg, 22%), identified by comparison of its ^{1}H NMR and IR data with those reported in the literature[20] {^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 122.8 (CH), 128.4 (CH), 129.2 (CH), 129.6 (CH), 129.7 (q), 130.5 (CH), 130.7 (CH), 131.6 (CH), 136.2 (CH), 136.3 (q), 145.6 (q). − MS (EI): *m*/*z* (%) = 251 [M^+] (25), 221 (25), 205 (85), 190 (25), 178 (35), 165 (20), 151 (15), 131 (29), 127 (50), 115 (100), 105 (85), 91 (95), 77 (90), 51 (55)}. This product was not present in the reaction mixture prior to column chromatography. (***ii***) Cinnamic aldehyde (**18**) (30 mg, 12%). (***iii***) A 2:1 diastereomeric mixture of 1-nitro-2-nitrooxy-1,4-diphenylbut-3-ene (**16**) (yellow oil) (35 mg, 6%) {^{1}H NMR (200 MHz, $CDCl_3$): δ_{major} = 5.28 (1 H, A part of an ABXY system, J_{AB} = 9 Hz, J_{AX} = 6.5 Hz, J_{AY} = 1 Hz), 5.45 (1 H, B part of an AB system, J_{AB} = 9 Hz), 5.85 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 6.5 Hz), 6.65 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 1 Hz), 7.20−7.55 (10 H); δ_{minor} = 4.65 (t, 1 H, *J* = 6.0 Hz), 4.95 (d, 1 H, *J* = 6.0 Hz), 6.20 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 6.0 Hz), 6.63 (d, 1 H, *J* = 16 Hz), 7.20−7.55 (10 H). − MS (positive-ion electron-impact method): *m*/*z* (%) = 251 (5), 178 (10), 161 (10), 133 (50), 131 (35), 115 (35), 105 (50), 103 (50), 91 (100), 77 (95), 65 (30), 51 (60). − MS (negative-ion electron-impact method): *m*/*z* (%) = 314 [M^-] (2), 252 (85), 178 (10), 147 (100), 46 (65). − IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 1650, 1555 (vs), 1535, 1490, 1450, 1365, 1310, 1280}. Elemental analysis was not performed in view of the fact that it proved impossible to obtain a pure sample owing to partial decomposition on the silica gel column and on standing in air. Compound **16** was treated with 10% aq. NaOH for 1 h; the reaction mixture was then extracted with diethyl ether, the organic phase was separated, and the solvent was evaporated. ^{1}H NMR and TLC analyses of the residue showed the disappearance of the starting **16** and the exclusive formation of cinnamic aldehyde (**18**). A further portion of compound **16** was absorbed onto silica gel for 24 h and then extracted with diethyl ether. ^{1}H NMR and TLC analyses showed the disappearance of the starting **16** and the exclusive formation of an 80:20 mixture of cinnamic aldehyde (**18**) and nitrodiene **19**. (***iv***) 1-Nitro-4-nitrooxy-1,4-diphenylbut-2-ene (**17**) (yellow oil) (170 mg, 27%) {^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.22 (0.35 H, *J* = 5.9 Hz; lines broadened by coupling to the signal at δ = 6.44; collapsing to a broad singlet upon irradiation at δ = 5.9), 5.24 (0.65 H, *J* = 5.3 Hz; lines broadened by coupling to the signal at δ = 6.44; collapsing to a broad singlet upon irradiation at δ = 5.9), 5.90 (m, 2 H), 6.44 (dd, 1 H, J_1 = 15.0 Hz, J_2 = 7.9 Hz; lines broadened by coupling to the signal at δ = 5.23; collapsing to a broad singlet upon irradiation at δ = 5.9), 7.25−7.55 (10 H). − MS (positive-ion electron-impact method): *m*/*z* (%) = 265 (10), 133 (10), 131 (20), 115 (15), 105 (90), 103 (35), 91 (30), 77 (100), 51 (50). − MS (negative-ion electron-

impact method): *m/z* = 314 [M⁻] (1), 252 (25), 237 (15), 147 (30), 121 (10), 46 (100). – IR ($CHCl_3$): $\tilde{\nu}_{max}$ =1550 (vs), 1535, 1490, 1450, 1360, 1280, 900 cm^{-1}}. Compound **17** was treated with 10% aq. NaOH for 1 h; the reaction mixture was then extracted with diethyl ether, the organic phase was separated, and the solvent was evaporated. ^1H NMR and TLC analyses of the residue showed the disappearance of the starting **17** and the exclusive formation of nitrodiene **19**. A further portion of compound **17** was absorbed onto silica gel for 24 h and then extracted with diethyl ether. ^1H NMR and TLC analyses showed the disappearance of the starting **17** and the exclusive formation of nitrodiene **19**. (***v***) A 60:40 inseparable mixture of diastereoisomeric 1,2-dihydroxy-4-(nitrophenyl)-1-phenylbut-3-ene (**15**) (light-yellow oil) (75 mg, 13%) {^1H NMR (200 MHz, $CDCl_3$): δ_{major} = 2.50 (br. s, 2 H, OH), 4.45 (ddd, 1 H, J_1 = 6.8 Hz, J_2 = 5.0 Hz, J_3 = 1.2 Hz), 4.80 (d, 1 H, J = 5.0 Hz), 6.15 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 6.8 Hz), 6.58 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 1.2 Hz), 7.10–7.50 (m, 9 H); δ_{minor} = 2.50 (br. s, 2 H, OH), 4.37 (ddd, 1 H, J_1 = 6.8 Hz, J_2 = 5.8 Hz, J_3 = 1.2 Hz), 4.58 (d, 1 H, J = 6.8 Hz), 6.05 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 5.8 Hz), 6.54 (dd, 1 H, J_1 = 16.0 Hz, J_2 = 1.2 Hz), 7.10–7.50 (m, 9 H). – MS (positive-ion electron-impact method): *m/z* (%) = 134 (65), 133 (95), 115 (40), 107 (75), 105 (35), 79 (75), 77 (100). MS (negative-ion electron-impact method): *m/z* (%) = 285 [M⁻] (10), 251 (25), 237 (25), 219 (15), 178 (95), 153 (25), 147 (45), 123 (40), 46 (100). – IR ($CHCl_3$): $\tilde{\nu}_{max}$ = 3560 and 3400 (br, OH), 1550, 1530, 1490, 1450, 1330 cm^{-1}. – $C_{16}H_{15}NO_4$ (285.3): calcd. C 67.36, H 5.30, N 4.91; found C 67.30, H 5.35, N 4.88}. ^1H NMR analysis of the mixture obtained from a reaction carried out under nitrogen showed the presence of starting diene **14** (90% yield) together with minor amounts of products **15**–**18** and other unidentified products (10% overall yield).

Reaction of *p*-Cresol (27): The reaction was performed at pH = 0 (6.0 mL of 1.2 M hydrochloric acid added) under nitrogen. ^1H NMR analysis of the reaction mixture using acetophenone (0.60 mmol) as an internal standard showed the exclusive formation of 4-methyl-2-nitrophenol (**28**)[21] in 90% yield based on the amount of *p*-cresol used. Identical results were obtained when the same reaction was carried out under air. This reaction was also carried out under nitrogen at pH = 7. ^1H NMR analysis of the reaction mixture using acetophenone (0.6 mmol) as an internal standard showed the exclusive formation of 4-methyl-2-nitrophenol (**28**) in 4% yield based on the starting *p*-cresol.

Reactions of Styrene and *p*-Cresol (27) with Nitric Oxide. – General Procedure: Styrene or *p*-cresol (**27**) (1.0 mmol) was dissolved in either acetonitrile (14 mL) acidified with 12 M hydrochloric acid (0.30 mL) (Table 3, conditions A), in neat acetonitrile (14 mL) (Table 3, conditions B), in a 5:2 mixture of acetonitrile/1 M hydrochloric acid (14 mL) (Table 3, conditions C), or in a 5:2 mixture of acetonitrile/water (14 mL) (Table 3, conditions D). The solutions were cooled to 0 °C and nitric oxide was bubbled through them for 60 min. After this time, TLC analysis showed almost complete disappearance of the starting styrene or *p*-cresol. The reaction mixtures were then extracted with diethyl ether, the organic layer was washed twice with water, and the solvent was evaporated.

Reaction of Styrene: The reaction mixtures obtained under conditions A, B, C, and D were analyzed by ^1H NMR using acetophenone (0.30 mmol) as an internal standard to determine the yields of the reaction products **1**, **2**, **4**, and **5**. The relative yields thus obtained and the overall yield, which is based on the starting styrene, are reported in Table 3. In the case of the reaction carried out in neat acetonitrile, the mixture was chromatographed on a silica gel column to separate β-nitrostyrene (**5**) (65 mg, 45%), which was identified by comparison with an authentic specimen.

Reaction of *p*-Cresol (27): The reaction mixtures obtained under conditions A and B were analyzed by ^1H NMR using acetophenone (0.30 mmol) as an internal standard. In both cases, 4-methyl-2-nitrophenol (**28**) was found to be present in 90% yield.

Acknowledgments

This work was financially supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST), Rome (funds 60% and 40%), and by the University of Bologna (funds for selected research topics A. A. 1999–2001). We gratefully thank Mr. Luca Zuppiroli for obtaining the mass spectra.

[1] In partial fulfilment of the requirements for the Ph.D. degree in Chemical Sciences, University of Bologna.
[2] The term "peroxynitrite" has been used throughout this paper to indicate both the unprotonated and the protonated forms. PN and HPN have been used to specifically indicate the $ONOO^-$ and ONOOH forms, respectively.
[3] [3a] J. M. Fukuto, L. J. Ignarro, *Acc. Chem. Res.* **1997**, *30*, 149–152. – [3b] S. Pfeiffer, B. Mayer, B. Hemmens, *Angew. Chem. Int. Ed.* **1999**, *38*, 1714–1731.
[4] W. A. Pryor, G. L. Squadrito, *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **1995**, *268*, L699–L722.
[5] [5a] J. W. Coddington, J. K. Hurst, S. V. Lymar, *J. Am. Chem. Soc.* **1999**, *121*, 2438–2443. – [5b] G. R. Hodges, K. U. Ingold, *J. Am. Chem. Soc.* **1999**, *121*, 10695–10701.
[6] [6a] W. A. Pryor, X. Jin, G. L. Squadrito, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11173–11177. – [6b] S. Goldstein, G. L. Squadrito, W. A. Pryor, G. Czapski, *Free Radicals Biol. Med.* **1996**, *21*, 965–974.
[7] R. Radi, J. S. Beckman, K. M. Bush, B. A. Freeman, *J. Biol. Chem.* **1991**, *266*, 4244–4250.
[8] H. Rubbo, R. Radi, M. Trujillo, R. Telleri, B. Kalyanaraman, S. Barnes, M. Kirk, B. A. Freeman, *J. Biol. Chem.* **1994**, *269*, 26066.
[9] L. Grossi, P. C. Montevecchi, S. Strazzari, *Eur. J. Org. Chem.*, in press.
[10] [10a] M. L. Scheinbaum, *J. Org. Chem.* **1970**, *35*, 2790–2792. – [10b] M. M. Baum, E. H. Smith, *J. Chem. Soc., Perkin Trans. 1* **1993**, 2513–2519.
[11] H. Suzuki, T. Mori, *J. Org. Chem.* **1997**, *62*, 6498–6502.
[12] Nitrous anhydride is a well-known nitrosating agent for thiols; see refs.[3b,4,13a] and: G. R. Upchurch, G. N. Welch, J. Loscalzo, *Adv. Pharmacol.* **1995**, *34*, 343–349; R. S. Lewis, S. R. Tannenbaum, W. M. Deen, *J. Am. Chem. Soc.* **1995**, *117*, 3933–3939.
[13] [13a] J. S. Stamler, D. I. Simon, J. A. Osborne, M. E. Mullins, D. Jaraki, T. Michel, D. J. Singel, J. Loscalzo, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 444–448. – [13b] L. Grossi, S. Strazzari, *J. Org. Chem.* **1999**, *64*, 8076–8079.
[14] S. Padmaja, R. E. Huie, *Biochem. Biophys. Res. Commun.* **1993**, *195*, 539–544.
[15] Neither products deriving from oxidation at the allylic position were obtained.
[16] The following ionization potentials (IP) have been reported in the literature: styrene: 8.46 eV (J. M. Dyke, H. Ozeki, M. Takahashi, M. C. R. Cockett, K. Kimura, *J. Chem. Phys.* **1992**, *97*, 8926–8933); benzalacetone: 8.80 eV (B. Schaldach, B. Grotemeyer, J. Grotemeyer, H.-F. Gruetzmacher, *Org. Mass Spectrom.* **1981**, *16*, 410–415); 1,4-diphenylbutadiene: 8.05 eV (C. Ruecker, D. Lang, J. Sauer, H. Friege, R. Sustmann, *Chem. Ber.* **1980**, *113*, 1663–1690); *p*-cresol: 8.22 eV (Y. Yagci, W. Schnabel, A. Wilpert, J. Bending, *J. Chem. Soc., Faraday Trans.* **1994**, *90*, 287–292); acetophenone: 9.51 eV (G. Pfister-Guillouzo, S. Geribaldi, J.-F. Gal, *Can. J. Chem.* **1982**, *60*, 1163–1172).
[17] R. Cornubert, P. Louis, *Bull. Soc. Chim. Fr.* **1938**, *5*, 520–534.
[18] R. Miravalles, A. J. Guillarmod, *Helv. Chim. Acta* **1966**, *49*, 2313–2320.

[19] M. N. Hughes, H. G. Nicklin, *J. Chem. Soc. A* **1968**, 450−452.
[20] P. Horsewood, G. W. Kirby, R. P. Sharma, J. G. Sweeny, *J. Chem. Soc., Perkin Trans. 1* **1981**, 1802−1806.
[21] J.-M. Poirer, C. Vottero, *Tetrahedron* **1989**, *45*, 1415−1422.
Received June 19, 2000
[O00310]