On the Role of Nitrogen Monoxide (Nitric Oxide) in the Nitration of a Tyrosine Derivative and Model Compounds

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The nitration of tyrosine derivatives with nitrogen monoxide (nitric oxide) occurs only in the presence of dioxygen, and the hypothesized mechanism involves nitrogen dioxide ($^{\cdot}NO_2$). For better understanding of the reaction mechanism, the nitration of model compounds – such as 1- and 2-naphthols and their corresponding 2- and 1-nitroso derivatives – with nitrogen monoxide in the presence and in the absence of dioxygen was studied. The results described here show that tyrosine and naphthols do not undergo nitrosation when they react with $^{\cdot}NO$, and so nitrosation of tyrosine in biological systems is highly unlikely. In addition, the oxidation of nitrosonaphthols $\rightleftarrows$ isonitrosonaphthols by nitric oxide and its derivatives to the corresponding nitro derivatives does not involve the oxoammonium ion, as reported previously. The mechanistic proposals are supported mainly by ESR investigation and electrochemical data.

Introduction

The formation of nitrotyrosine in proteins and tissues during oxidative stress takes place in a number of pathologies such as lung inflammation,[1,2] atherosclerosis,[3,4] aging,[5] infections,[6] and others. In most cases, the presence of this molecule is attributed to the action of peroxynitrite or peroxynitrous acid, formed by interaction of nitric oxide with superoxide[7–9] and dioxygen.[10–12]

The formation of nitrotyrosine was tentatively explained by the action of nitrogen monoxide and peroxidase enzyme intermediates.[13] The proposed mechanism is based on the formation of nitrosotyrosine by coupling of the phenoxyl radical with nitric oxide and on the oxidation of this isonitroso derivative to the corresponding oxoammonium ion, which then affords the nitro derivative through addition of water.[13] Nitrogen monoxide itself is a very weak hydrogen abstractor and is not able to abstract hydrogen from phenols as erroneously described;[14,15] this reaction cannot occur because of the high endothermicity of the process: the bond dissociation enthalpy (BDE) of H–NO is 208.58 kJ/mol[16] and that of the O–H of phenols is higher than 334 kJ/mol.[17] In addition, phenoxyl radicals cannot be formed by an oxidative pathway, due to the low reduction potential of the $^{\cdot}NO/NO^-$ couple ($E = -0.80$ V in water vs. SCE).[18] $^{\cdot}NO$ derivatives are more reactive: peroxynitrite ($ONOO^-$) is, in fact, a strong oxidant ($E^{\circ}_{ONOO^{\cdot}/^{\cdot}NO2} = 1.60$ V in water)[19,20] and nitrosodioxidanyl ($ONOO^{\cdot}$) could act as hydrogen abstractor, the BDE of H–ONOO being 423.22 kJ/mol, but is not a very good oxidant: the reduction potential for the $ONOO^{\cdot}/ONOO^-$ couple is $E_{1/2} = 0.35 \pm 0.02$ V vs. SSCE.[21] Nitrosodioxidanyl rapidly reacts with $^{\cdot}NO$ to afford two molecules of $^{\cdot}NO_2$. In addition, $^{\cdot}NO_2$ and N_2O_3, formed as shown in Equations (1)–(4) (Scheme 1), are much more reactive than $^{\cdot}NO$: they are both strong oxidants ($E^{\circ}_{^{\cdot}NO2/NO2^-} = 0.99$ V; $E^{\circ}_{N2O3/^{\cdot}NO,NO2^{\cdot}-} = 0.80$ V, both in water),[22,23] and $^{\cdot}NO_2$ is a good hydrogen abstractor, too. In fact, the bond dissociation enthalpy of the H–ONO bond is 327.46 kJ/mol.[16]

$$^{\cdot}NO + O_2 \rightleftharpoons {}^{\cdot}ONOO \quad (1)$$

$$^{\cdot}ONOO + {}^{\cdot}NO \longrightarrow ONOONO \quad (2)$$

$$ONOONO \longrightarrow 2\ {}^{\cdot}NO_2 \quad (3)$$

$$^{\cdot}NO + {}^{\cdot}NO_2 \rightleftharpoons N_2O_3 \quad (4)$$

Scheme 1

In order to demonstrate whether the nitration of tyrosine could occur through the action of $^{\cdot}NO$ or its other derivatives, we studied the nitration of *N*-benzoyltyrosine ethyl ester (**1**) and of similar compounds such as 1-naphthol (**2a**) and 2-naphthol (**2b**) with $^{\cdot}NO$, in the presence and in the absence of dioxygen, and with $^{\cdot}NO_2$. The corresponding nitroso derivatives **3a** and **3b** were also tested under the

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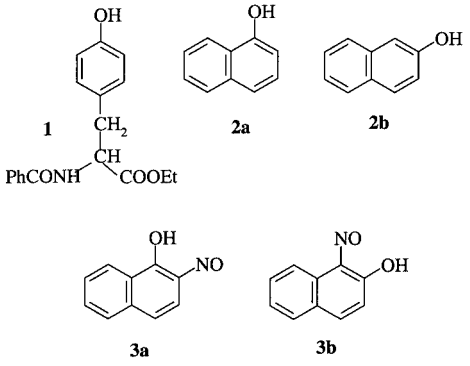
same experimental conditions to verify the possible involvement of these nitroso species in the nitration reaction. The nitroso derivative of **1** was not used, due to the difficulty involved in synthesizing this compound.



The results reported here demonstrate that nitrogen monoxide is not able to nitrosate tyrosine by itself. In addition, from the electrochemical data obtained for model compounds, it may be deduced that an isonitroso compound would be unlikely to be transformed into the corresponding nitro derivative by any radical derived from ·NO because of the high oxidation potential of the iminoxyl radical involved.

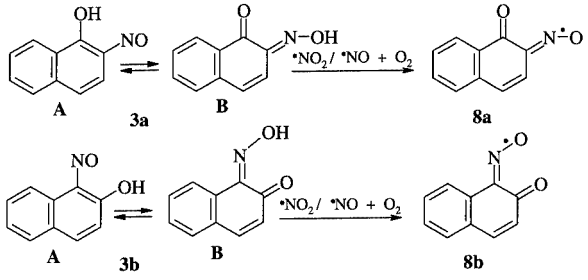
Results

Formation of Phenoxyl and Iminoxyl Radicals, Detected by ESR

All experiments were carried out with ·NO gas both in the presence and in the absence of dioxygen. Previously, Janzen[14] had described the phenoxyl radical of 2,4,6-tri-*tert*-butylphenol, obtained by bubbling ·NO into a cyclohexane solution of the phenol. Attempts to obtain the same result by addition of ·NO gas, either in the absence or in the presence of dioxygen, or by addition of $\cdot NO_2$ to a benzene solution of 2,4,6-tri-*tert*-butylphenol, failed, whereas an ESR signal was obtained on treating the same solution with PbO_2.

Similar results were obtained when starting from *N*-benzoyltyrosine ethyl ester (**1**). Because **1** reacts with $\cdot NO_2$ to give the nitro derivative **6** in almost quantitative yield, the failure to detect the ESR signal could be attributable to the fast coupling of $\cdot NO_2$ with the phenoxyl radical at the carbon position (Scheme 2).

Not even the naphthoxyl radicals of **2a** and **2b** were ever detected in the presence of ·NO either in the absence or in the presence of dioxygen. However, the spectra of the iminoxyl radicals **8a** and **8b** were recorded when benzene solutions of 2-nitroso-1-naphthol (**3a**) and 1-nitroso-2-naphthol (**3b**) were treated with ·NO in the presence of dioxygen (Scheme 3). Iminoxyl radicals **8a** and **8b** were also obtained when benzene solutions of **3a** and **3b** were allowed to react with benzene solutions of $\cdot NO_2$.



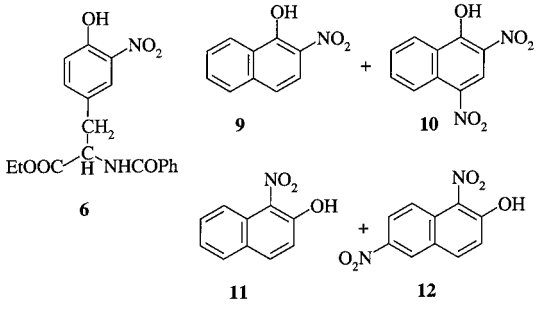
Scheme 3

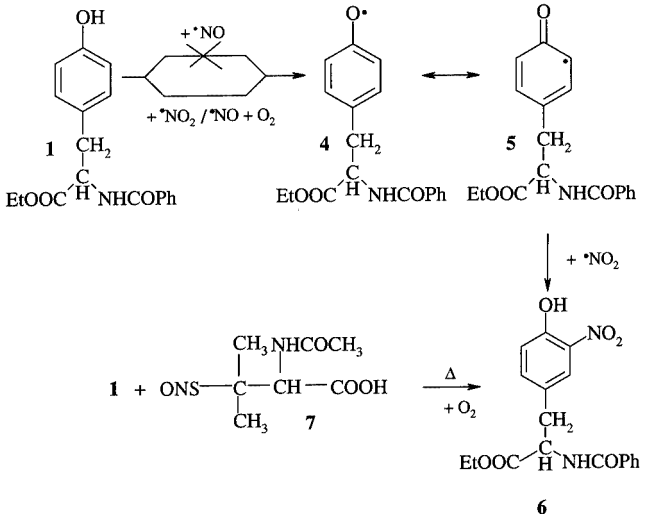
The same results were obtained upon oxidation of benzene solutions of **3a** and **3b** with PbO_2; the hyperfine coupling constants of **8a** and **8b** (see Exp. Sect.) are in agreement with those reported in the literature.[24]

Macroscale Reactions of Compounds 1, 2a, 2b, 3a, and 3b with ·NO and $\cdot NO_2$

·NO was used directly as a gas or generated by thermal decomposition of *N*-acetyl-*S*-nitroso-D,L-penicillamine (**7**), and all experiments were carried out both in the absence and in the presence of dioxygen. $\cdot NO_2$ was generated by thermal decomposition of $Pb(NO_3)_2$ (see Exp. Sect.).

From the reactions of **1**, **2a**, **2b**, **3a**, and **3b** with ·NO in the absence of dioxygen, only mononitro compounds were recovered, with low conversion factors (Runs 1, 6, 10, 16, and 20, Table 1). In the presence of dioxygen, however, the yields of nitro compounds were significantly higher (Runs 2, 7, 11, 17, and 21, Table 1).





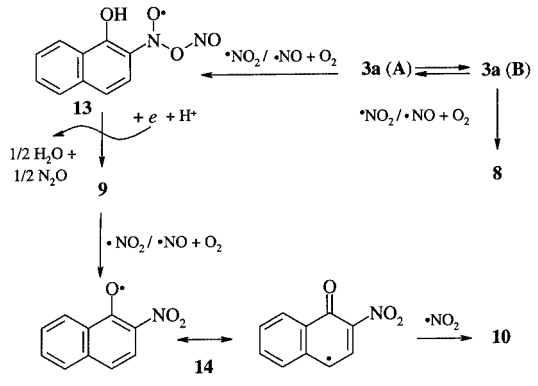
Scheme 2

Table 1. Conversion factors and yields of isolated products for the reactions between compounds **1**, **2a**, **2b**, **3a**, and **3b** and ·NO gas or **7**, in the absence and in the presence of dioxygen, and with $^{\cdot}NO_2$ in 1:1.1 and 1:1.3 ratios

Run	Reagents	Conversion factor [%]	Isolated product (Yield [%])
1	**1** + ·NO	8	**6** (95)
2	**1** + ·NO + O_2	100	**6** (92)
3	**1** + **7**	0	–
4	**1** + **7** + O_2	40	**6** (97)
5	**1** + $^{\cdot}NO_2$ (1:1.1)	100	**6** (97)
6	**2a** + ·NO	15	**9** (96)
7	**2a** + ·NO + O_2	100	**9** (55), **10** (45)
8	**2a** + $^{\cdot}NO_2$ (1:1.1)	100	**9** (95)
9	**2a** + $^{\cdot}NO_2$ (1:3)	100	**10** (90)
10	**2b** + ·NO	12	**11** (94)
11	**2b** + ·NO + O_2	100	**11** (57), **12** (43)
12	**2b** + **7**	0	–
13	**2b** + **7** + O_2	30	**11** (97)
14	**2b** + $^{\cdot}NO_2$ (1:1.1)	100	**11** (98)
15	**2b** + $^{\cdot}NO_2$ (1:3)	100	**12** (96)
16	**3a** + ·NO	17	**9** (96)
17	**3a** + ·NO + O_2	100	**9** (43), **10** (57)
18	**3a** + $^{\cdot}NO_2$ (1:1.1)	100	**9** (70)
19	**3a** + $^{\cdot}NO_2$ (1:3)	100	**10** (77)
20	**3b** + ·NO	10	**11** (97)
21	**3b** + ·NO + O_2	100	**11** (32), **12** (68)
22	**3b** + **7**	0	–
23	**3b** + **7** + O_2	10	**11** (100)
24	**3b** + $^{\cdot}NO_2$ (1:1.1)	100	**11** (82)
25	**3b** + $^{\cdot}NO_2$ (1:3)	100	**12** (75)

In the reactions carried out with ·NO in the absence of dioxygen, the presence of nitro derivatives, albeit in low yields, was probably attributable to the incomplete elimination of dioxygen when gaseous ·NO was used. When the reaction was carried out by mixing thoroughly degassed solutions of **1** and **7** under vacuum, no traces of **6** were observed (Run 3, Table 1), whereas that compound was isolated in the presence of dioxygen (Run 4, Table 1). Compounds **2b** and **3b** also afforded the nitro derivative **11** (Runs 13 and 23, Table 1) when treated with **7** in the presence of dioxygen, whereas no reaction was observed in its absence (Runs 12 and 22, Table 1). In all the reactions carried out with **7**, *N*-acetyl-D,L-penicillamine disulfide was recovered quantitatively.

Compounds **1**, **2a**, **2b**, **3a**, and **3b**, on treatment with $^{\cdot}NO_2$ in 1:1.1 molar ratio, afforded the mononitro derivatives **6**, **9**, and **11**, respectively, in very high yields (Runs 5, 8, 14, 18, and 24; Table 1), whereas when compounds **2a**, **2b**, **3a**, and **3b** were allowed to react with an excess of $^{\cdot}NO_2$ (1:3 molar ratio), only the dinitrated products **10** and **12** were isolated (Runs 9, 15, 19 and 25; Table 1) (Scheme 4).



Scheme 4

Discussion

From the results obtained, it is clear that ·NO is unable, in the absence of dioxygen, to oxidize the phenolic OH group to generate the phenoxyl radical either by transfer of an electron and a proton (this mechanism could be ruled out on the basis of the redox potentials of the reagents) or by hydrogen abstraction, as demonstrated by the ESR experiments. In fact, nitrosation of tyrosine **1** and of the other compounds **2a** and **2b** by ·NO alone does not occur. ·NO is not a very reactive species towards organic substrates,[25] but it rapidly reacts with dioxygen to form the nitrosodioxidanyl radical (ONOO·), as shown in Equation (1).[10–12] ONOO· may be involved in the generation of the phenoxyl radicals from phenols, but its most likely fate is the reaction with ·NO at a diffusion-controlled rate to form $^{\cdot}NO_2$,[26] which could be the true hydrogen abstractor. The intermediate formation of phenoxyl **4/5** generated by $^{\cdot}NO_2$ hydrogen abstraction from tyrosine **1** and subsequent coupling with another molecule of $^{\cdot}NO_2$ (Scheme 2) results in its nitration. A similar mechanism may also be invoked for naphthols **2a** and **2b**. N_2O_3 [Scheme 1, Equation (4)] may compete with $^{\cdot}NO_2$ in the mononitration of **1**, **2a**, and **2b**, to afford nitro derivatives **6**, **9**, and **11**, respectively, but the intermediate formation of N_2O_3 from coupling between

·NO and $·NO_2$ has not yet been fully demonstrated.[27,28] Moreover, the proposed mechanism through $·NO_2$ is in agreement with that recently reported for the aromatic nitration of phenolic *S*-nitrosothiols in nonaqueous aerobic media.[29]

It is noteworthy that nitrosotyrosine and nitrosonaphthols were never obtained in the reactions with ·NO, while when 2-substituted and 1,2-disubstituted indoles were treated under the same conditions, the corresponding nitroso derivatives were always isolated, whether in the absence or in the presence of dioxygen.[30]

The macroscale reactions are in agreement with the ESR experiments; in fact, the nitro derivatives are formed in high yields with ·NO only in the presence of dioxygen (see Table 1). Because the same results were obtained both with $·NO/O_2$ and with $·NO_2$, it may probably be assumed that the latter is responsible for nitration.

Similarly, nitrosonaphthols **3a** and **3b** react with ·NO only in the presence of O_2, or with $·NO_2$. In agreement with the well-known spin-trapping ability of nitroso compounds,[31] 2-nitroso-1-naphthol (**3a**, **A**) could react with $·NO_2$ to give the spin adduct **13**. This could subsequently evolve to the nitro derivative **9** by hydrogen abstraction and elimination of HNO, which decomposes to form H_2O and N_2O (Scheme 4). This hypothesis was supported by the fact that when the two well-known spin traps α-*tert*-butylphenylnitrone (PBN) and 5,5-dimethylpyrrolidine 1-oxide (DMPO) were treated with $·NO_2$, the corresponding acyl nitroxides were detected by ESR.[32] The dinitro compound **10** could be obtained through the intermediate formation of the nitronaphthoxyl **14**, as shown in Scheme 4. In order to gain more information on the oxidation of the nitroso to the corresponding nitro group, a benzene solution of nitrosobenzene was treated with $·NO_2$ in the absence of dioxygen; under these conditions, nitrosobenzene was quantitatively transformed into nitrobenzene. $·NO_2$ is not able to oxidize the nitrosobenzene by electron transfer,[33] while it may be trapped by the nitroso group through the oxygen atom, affording a spin adduct such as **13**, which spontaneously gives the nitro derivative.[31] Unfortunately, in the case of **3a** (**A**) all attempts to detect the signal of **13** failed.

The formation of **11** and **12** from **3b** could also be explained in the same way as shown in Scheme 4 for **9** and **10**. As can be seen in Table 1, the production of the dinitro derivatives is due to the large excess of ·NO; indeed, when ·NO was present in a roughly stoichiometric ratio (i.e., when it is generated by thermal decomposition of **7** in the presence of dioxygen), only the mononitro derivatives were formed. The same can be said for $·NO_2$.

In order to verify the possible transformation of nitrosophenols into the corresponding nitro derivatives by ·NO via an oxoammonium ion as previously reported for tyrosine,[13] the oxidation potentials of nitrosonaphthols **3a** and **3b** were measured (see Table 2). According to the values obtained, it is highly unlikely that nitrosonaphthols could be oxidized to their iminoxyl radicals **8a** and **8b**[34,35] and thereafter to the corresponding oxoammonium ions, ·NO being both a very bad hydrogen abstractor and a very poor oxidant (see above). The iminoxyl radicals were obtained only when **3a** and **3b** were treated with ·NO in the presence of dioxygen or directly with $·NO_2$. On the basis of their redox potentials, the formation of oxoammonium ions from iminoxyl radicals cannot take place even with $·NO_2$.[33,34]

Table 2. Electrochemical data of nitrosonaphthols **3a** and **3b** (1×10^{-3} mol L^{-1}) in dried CH_3CN/TBAEFF (0.1 mol L^{-1}) at a platinum working electrode; reported potentials are vs. $Ag/AgClO_4$ 0.1 mol L^{-1} – CH_3CN/fritted glass disk/TBAEFF 0.1 mol L^{-1} – CH_3CN/fritted glass disk; scan rate 200 mV s^{-1}

Substrate	E_{pa1} [V]	i/c ($\times 10^3$)	E_{pa2} [V]	i/c ($\times 10^3$)
3a	+1.52	4.90	+1.83	4.60
3b	+1.64	4.82	+2.06	13.24

The results described here were obtained with model compounds of tyrosine, and so cannot be extrapolated to reactions occurring in vivo, either in terms of the reaction conditions (medium and possible enzymatic catalysis) or of the reagents' concentrations.

Experimental Section

General: Melting points were determined with an Electrothermal apparatus. EPR spectra were recorded with a Varian E4 spectrometer (containing a ruby in the cavity as reference) interfaced with a PC. ^1H NMR spectra were recorded at room temperature in $CDCl_3$ solution with a Varian Gemini 200 spectrometer (TMS as reference peak). Mass spectra were obtained with a Carlo Erba QMD1000 mass spectrometer equipped with a Fisons GC 8060 gas chromatograph. *N*-Benzoyltyrosine ethyl ester (**1**), 1-naphthol (**2a**), 2-naphthol (**2b**), 2-nitroso-1-naphthol (**3a**), 1-nitroso-2-naphthol (**3b**), 2-nitro-1-naphthol (**9**), 2,4-dinitro-1-naphthol (**10**), *N*-acetyl-D,L-penicillamine, and nitrogen monoxide were purchased from Aldrich; *N*-acetyl-*S*-nitroso-D,L-penicillamine (**7**) was prepared according to the literature.[36] All the other reagents and solvents were Carlo Erba or Aldrich RP-ACS grade and were purified according to the literature.[37]

EPR Measurements: All the experiments were carried out directly in the ESR cavity by use of an inverted U cell as reported in the literature.[38]

$·NO_2$ Benzene Solution: $·NO_2$, generated by thermal decomposition of $Pb(NO_3)_2$, was bubbled into cooled benzene (previously degassed under a stream of argon) until the solution turned yellow. The concentration of $·NO_2$ was determined each time by weighing the solution before and after bubbling.

Phenoxyl Radical of 2,4,6-Tri-*tert*-butylphenol and PbO_2: 2,4,6-Tri-*tert*-butylphenol solution in dried cyclohexane (4.5×10^{-3} mol L^{-1}, 2 mL) was placed in one of the two legs of an inverted U cell, and PbO_2 (10 mg suspended in 2 mL of dried cyclohexane) was placed in the other leg. The two solutions were well degassed under a stream of argon, mixed, transferred into the aqueous cell, and placed in the ESR cavity. A well-resolved ESR signal was detected and attributed to the phenoxyl radical: $a_H = 1.68$ (2 H) Gauss.

Phenoxyl Radical of 2,4,6-Tri-*tert*-butylphenol with ·NO in the Absence and in the Presence of Dioxygen or with $\cdot NO_2$: 2,4,6-Tri-*tert*-butylphenol solution in dried cyclohexane (4.5×10^{-3} mol L^{-1}, 2 mL) was placed in one leg of an inverted U cell and thoroughly degassed under a stream of argon for 5 min. Dried and degassed cyclohexane (2 mL) was subjected to bubbling with ·NO for 2 min in the other leg of the U cell. The two solutions were mixed, transferred into the aqueous cell, and placed in the ESR cavity. No ESR signal was detected, neither when the same solution was exposed to air, nor when the solution of the phenol was treated with a solution of $\cdot NO_2$.

Phenoxyl Radical of *N*-Benzoyltyrosine Ethyl Ester (1) with ·NO in the Absence and in the Presence of Dioxygen: The experiments were carried out under the same conditions as described above. No ESR signal was detected, nor when the same solution was exposed to the air.

Iminoxyl Radicals of 2-Nitroso-1-naphthol (3a) and 1-Nitroso-2-naphthol (3b) with PbO_2: A solution of the nitrosonaphthol in dried benzene (4.5×10^{-3} mol L^{-1}, 2 mL) in one leg of an inverted U cell, and PbO_2 (10 mg suspended in 2 mL of dried benzene) in the other leg, were degassed under a stream of argon, mixed, transferred into the aqueous cell, and placed in the ESR cavity. ESR signals of the iminoxyl radicals **8a** and **8b**, obtained from **3a** and **3b**, respectively, were detected: **8a**, $a_N = 26.26$, $a_H = 1.71$ (1 H), $a_H = 0.79$ (2 H) Gauss; **8b**, $a_N = 26.98$, $a_H = 0.48$ (2 H) Gauss.

Iminoxyl Radicals of 2-Nitroso-1-naphthol (3a) and 1-Nitroso-2-naphthol (3b) with ·NO in the Absence and in the Presence of Dioxygen: A solution of nitrosonaphthol in dried cyclohexane (4.5×10^{-3} mol L^{-1}, 2 mL) in one leg of an inverted U cell was thoroughly degassed under a stream of argon for 5 min. Previously degassed dried cyclohexane (2 mL) was subjected to ·NO bubbling for 2 min in the other leg of the U cell. The two solutions were mixed, transferred into the aqueous cell, and placed in the ESR cavity. No ESR signal was detected in the absence of dioxygen, but when the same solutions were exposed to air, intense and well-resolved signals of iminoxyl radicals **8a** and **8b** were recorded. No significant changes in the hyperfine coupling constants, reported above, were observed.

Iminoxyl Radicals of 2-Nitroso-1-naphthol (3a) and 1-Nitroso-2-naphthol (3b) with $\cdot NO_2$: A well-degassed solution of nitrosonaphthol in dried benzene (4.5×10^{-3} mol L^{-1}, 2 mL) in one leg of an inverted U cell, and dried $\cdot NO_2$ benzene solution (2.2×10^{-3} mol L^{-1}, 2 mL) in the other leg of the U cell, were mixed, transferred into the aqueous cell, and placed in the ESR cavity. Intense and well-resolved signals of iminoxyl radicals **8a** and **8b** were recorded.

Macroscale Reactions

Treatment of *N*-Benzoyltyrosine Ethyl Ester (1), 1-Naphthol (2a), 2-Naphthol (2b), 2-Nitroso-1-naphthol (3a), and 1-Nitroso-2-naphthol (3b) with ·NO

In the Absence of O_2 − General Procedure: The reactions were carried out in a 100-mL round flask equipped with a three-directional tap and tubes connected to a vacuum pump and to cylinders of argon and of ·NO. In this apparatus, a solution of *N*-benzoyltyrosine ethyl ester (1.0 mmol in 10 mL of dried benzene) was degassed under vacuum and washed with a stream of argon. This procedure was repeated three times in order to eliminate dioxygen as much as possible. ·NO was then added to this solution, under vacuum at room temperature, until the external pressure was regenerated. The reaction mixture was left to react (magnetic stirring) at room temperature for 2 h and then quickly flushed with argon. The solvents were removed to dryness. The residue was chromatographed on an SiO_2 column (eluent: cyclohexane, to which ethyl acetate was progressively added until an 8:2 ratio was obtained). Together with the starting material, 2-nitro-*N*-benzoyltyrosine ethyl ester (**6**) was recovered (Table 1) and identified by its spectroscopic data (see below). The same procedure was applied for treatment of compounds **2a**, **2b**, **3a**, and **3b**. The isolated products, together with conversion factors and yields, are reported in Table 1. 2-Nitro-1-naphthol (**9**) and 2,4-dinitro-1-naphthol (**10**) were identified by comparison with authentic samples. 1-Nitro-2-naphthol (**11**) and 1,6-dinitro-2-naphthol (**12**) were identified by their spectroscopic data and by comparison with those reported in the literature (see below).

In the Presence of O_2 − General Procedure: ·NO was bubbled for 10 s through a solution of *N*-benzoyltyrosine ethyl ester (**1**, 1.0 mmol in 10 mL of dried benzene), previously saturated with O_2. The mixture was allowed to react (magnetic stirring) at room temperature for 2 h and then quickly flushed under a stream of argon. The solvents were removed to dryness. The residue was chromatographed on an SiO_2 column (eluent: cyclohexane, to which ethyl acetate was progressively added until an 8:2 ratio was obtained). 2-Nitro-*N*-benzoyltyrosine ethyl ester (**6**) was recovered in almost quantitative yield (Table 1) and identified by its spectroscopic data. The same procedure was applied for treatment of compounds **2a**, **2b**, **3a**, and **3b**. The isolated products, together with conversion factors and yields, are reported in Table 1. 2-Nitro-1-naphthol (**9**) and 2,4-dinitro-1-naphthol (**10**) were identified by comparison with authentic samples. 1-Nitro-2-naphthol (**11**) and 1,6-dinitro-2-naphthol (**12**) were identified by their spectroscopic data and by comparison with those reported in the literature (see below).

2-Nitro-*N*-benzoyltyrosine Ethyl Ester (7):[39] M.p. 160−162 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.32 (t, J = 7.2 Hz, 3 H, $-CH_3$), 3.26 (J = 13.9, J = 5.8 Hz, 2 H, qd, $-CH_2-$), 4.25 (q, J = 7.2 Hz, 2 H, $-CH_2O-$), 5.03 (td, J = 5.8, J = 1.5 Hz, 1 H, −CH−), 6.80 (br. s, 1 H, NH), 7.06 (d, J = 8.6 Hz, 1 H, 6-H), 7.44 (m, 5 H, arom), 7.75 (dd, J = 8.6, J = 2.0 Hz, 1 H, H_5), 7.90 (d, J = 2.0 Hz, 1 H, H_3), 10.47 (s, 1 H, OH) ppm. MS (EI^+): *m*/*z* (%) = 358 (12) [M^+], 340 (25), 122 (47), 105 (100), 77(65). $C_{18}H_{18}N_2O_6$ (358.35): calcd. C 60.33, H 5.06, N 7.82, O 26.79; found C 60.29, H 5.08, N 7.86, O 26.77.

1-Nitro-2-naphthol (11):[40] M.p. 104−106 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.27 (d, J = 9.1 Hz, 1 H, arom), 7.52 (td, J = 7.5, J = 1.1 Hz, 1 H, arom), 7.75 (td, J = 8.6, J = 1.4 Hz, 1 H, arom), 7.83 (d, J = 8.0 Hz, 1 H, arom), 8.02 (d, J = 9.1 Hz, 1 H, arom), 8.94 (d, J = 8.6 Hz, 1 H, arom), 12.20 (br. s, 1 H, −OH) ppm. MS (EI^+): *m*/*z* (%) = 189 (100) [M^+], 173 (12), 143 (25), 131 (37), 115 (51). $C_{10}H_7NO_3$ (189.17): calcd. C 63.50, H 3.73, N 7.40, O 25.37; found C 63.55, H 3.72, N 7.39, O 25.34.

1,6-Dinitro-2-naphthol (12):[41] M.p. 190−192 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.46 (d, J = 9.1 Hz, 1 H, H_7), 8.19 (d, J = 9.1 Hz, 1 H, H_8), 8.49 (dd, J = 9.6, J = 2.3 Hz, 1 H, H_4), 8.76 (d, J = 2.3 Hz, 1 H, H_5), 9.08 (d, J = 9.6 Hz, 1 H, H_3), 12.21 (br. s, 1 H, −OH) ppm. MS (EI^+): *m*/*z* (%) = 234 (100) [M^+], 204 (63), 158 (45), 114 (58). $C_{10}H_6N_2O_5$ (218.168): calcd. C 55.06, H 2.77, N 12.84, O 29.33; found C 54.98, H 2.79, N 12.86, O 29.37.

Treatment of 1-Naphthol (2a), 2-Naphthol (2b), 2-Nitroso-1-naphthol (3a), and 1-Nitroso-2-naphthol (3b) with $\cdot NO_2$. General Procedure

Substrate/$\cdot NO_2$ Ratio 1:1.1: $\cdot NO_2$ (1.1 mmol in 10 mL of dried benzene) was added to a previously degassed solution of 1-naphthol

(**2a**, 1.0 mmol in 10 mL of dried benzene). The reaction mixture was allowed to react (magnetic stirring) at room temperature for 2 h, and then concentrated to dryness. The residue was chromatographed on an SiO_2 column (eluent: cyclohexane, to which ethyl acetate was progressively added until an 8:2 ratio was obtained). 2-Nitro-1-naphthol (**9**) was obtained (Table 1). The same procedure was applied for treatment of compounds **2b**, **3a**, and **3b**; the isolated products, together with conversion factors and yields, are reported in Table 1. 2-Nitro-1-naphthol (**9**) was identified by comparison with an authentic sample, while 1-nitro-2-naphthol (**11**) was identified by its spectroscopic data.

Substrate/$^{\cdot}NO_2$ Ratio 1:3: The reactions were carried out as described above. Naphthol **2a** (1.0 mmol in 10 mL of dried benzene) was added to a solution of $^{\cdot}NO_2$ (3 mmol in 10 mL of dried benzene). 2,4-Dinitro-1-naphthol (**10**) was obtained (Table 1). The same procedure was followed for compounds **2b**, **3a**, and **3b**. The isolated products, together with conversion factors and yields, are reported in Table 1. 2,4-Dinitro-1-naphthol (**10**) was identified by comparison with authentic samples. 1,6-Dinitro-2-naphthol (**12**) was identified by its spectroscopic data.

Treatment of *N*-Benzoyltyrosine Ethyl Ester (1), 2-Naphthol (2b), and 1-Nitroso-2-naphthol (3b) with *N*-Acetyl-*S*-nitroso-D,L-penicillamine (7)

In the Absence of O_2. General Procedure: A solution of *N*-acetyl-*S*-nitroso-D,L-penicillamine (**7**, 3.0 mmol in 10 mL of methanol) was added to a solution of *N*-benzoyltyrosine ethyl ester (**1**, 1.0 mmol in 10 mL of dried benzene). The reaction mixture was degassed under a stream of argon and heated under reflux with magnetic stirring for 6 h. The residue was chromatographed on an SiO_2 column (eluent: cyclohexane, to which ethyl acetate was progressively added until an 8:2 ratio was obtained). Besides starting material, only *N*-acetyl-D,L-penicillamine disulfide was recovered, in almost quantitative yield (Table 1), and identified by its melting point: 127−129 °C (ref.[36] 127−129 °C).

In the Presence of O_2. General Procedure: A solution of *N*-acetyl-*S*-nitroso-D,L-penicillamine (**7**, 3.0 mmol in 10 mL of methanol) was added to a solution of *N*-benzoyltyrosine ethyl ester (**1**, 1.0 mmol in 10 mL of dried benzene). Dioxygen was bubbled into the solution and the reaction mixture was heated under reflux for 6 h. The residue was chromatographed on an SiO_2 column (eluent: cyclohexane, to which ethyl acetate was progressively added until an 8:2 ratio was obtained). Besides starting material, 2-nitro-*N*-benzoyltyrosine ethyl ester (**6**) and *N*-acetyl-D,L-penicillamine disulfide were recovered (Table 1) and identified by their spectroscopic data.

Electrochemistry: Cyclic voltammetry of nitrosonaphthols **3a** and **3b** (substrate concentration ca. 1×10^{-3} mol L^{-1}) was carried out at a platinum working electrode in dried CH_3CN containing tetrabutylammonium hexafluorophosphate (TBAEFF, 0.1 mol L^{-1}) as supporting electrolyte. A platinum wire was used as counter-electrode and $Ag/AgClO_4$ 0.1 mol L^{-1} − CH_3CN/fritted glass disk/TBAEFF 0.1 mol L^{-1} − CH_3CN/fritted glass disk as reference.[42] Experiments were performed with a three-electrode multipolarograph AMEL 472 coupled with a digital *x*/*y* recorder AMEL 863. 2,6-Lutidine was used as deprotonating agent.

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