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Radical Scavenging by *N*-Aminoazaaromatics

Takashi Itoh, Michiko Miyazaki, Hiromi Maeta, Yûji Matsuya,
Kazuhiro Nagata and Akio Ohsawa*

School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

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Abstract—*N*-Aminoazaaromatics were found to react with nitric oxide in the presence of oxygen to afford deaminated products in high yields. The reaction proceeded almost instantaneously in various solvents including water, and one to two equivalent of NO was consumed depending upon the amount of oxygen coexisted, and 1 equivalent of N_2O was released in the reaction. In addition, *N*-aminoazoles were deaminated by potassium superoxide to give parent azoles in good yields. Two equivalents of superoxide was consumed, and about half equivalents of both nitrite and nitrate ion were released. The results demonstrated that *N*-aminoazoles have ability to protect the biological system against the oxidation promoted by radicals such as nitrogen oxides and superoxide.

Introduction

Despite its simple structure, nitric oxide (NO) has been found to play diverse roles in biological systems.[1,2] Accompanied by the positive physiological roles, NO causes extensive damage to the body in the cases that NO is released at relatively higher concentration.[3] In these circumstances, NO is activated by the reaction with various active oxygens to afford more reactive nitrogen oxides, and the ultimate reactive species included in the processes has been an important target for toxicological studies.[4] From the viewpoint of environmental sciences, the conversion of NO into species such as N_2O and N_2 has important consequences.[5,6]

For the understanding of reactive species of nitrogen oxides toward organic molecules, we have recently been studying the reaction of nitric oxide with nitrogen compounds in the presence and absence of oxygen,[7–10] and found that oxygen plays two types of roles in this system, that is, one in which oxygen is consumed according to a stoichiometry, and the other where oxygen functions in a catalytic manner.[11,12] With these results in hand, we next applied the reaction system to development of a new methodology of nitric oxide quenching, and found that *N*-aminoazaaromatics reacted rapidly with NO in the presence of various amounts of oxygen to afford corresponding deaminated products in quantitative yields.[13] NO or higher nitrogen oxides was transformed stoichiometrically to nitrous oxide N_2O. In addition, *N*-aminoazole derivatives were also found to quench superoxide anion, which is a precursor of peroxynitrite[14, 15] and other active oxygen species.[16] This paper describes these results.

Results and Discussion

Reaction of *N*-aminoazaaromatics with nitric oxide in the presence of various amounts of oxygen

N-Aminopyrazole (**1**)[17] was allowed to react for 5 min with NO under air in acetonitrile to give pyrazole in 100% yield accompanied by 1 equivalent (equiv) of N_2O, whose quantitative formation was confirmed by gas chromatography (GC). Other *N*-aminated compounds of indazoles and imidazoles were deaminated in the same manner to give the products (Scheme 1 and Fig. 1). The yield was measured with ^{1}H NMR using mesitylene or 1,4-dioxane as an internal standard.

As shown in Table 1, an equimolar amount of NO afforded about 50% yield of the product, and the starting material was almost entirely consumed by 2 equiv of nitric oxide in a very short reaction time. Although the reaction time (5 min) was adopted to obtain the reproducible data, the reaction completed instantaneously, which was indicated by TLC. Unexpectedly, the same amount of NO is consumed even when the substrate has a positive charge (substrates **10**–**13**).

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*Corresponding author.

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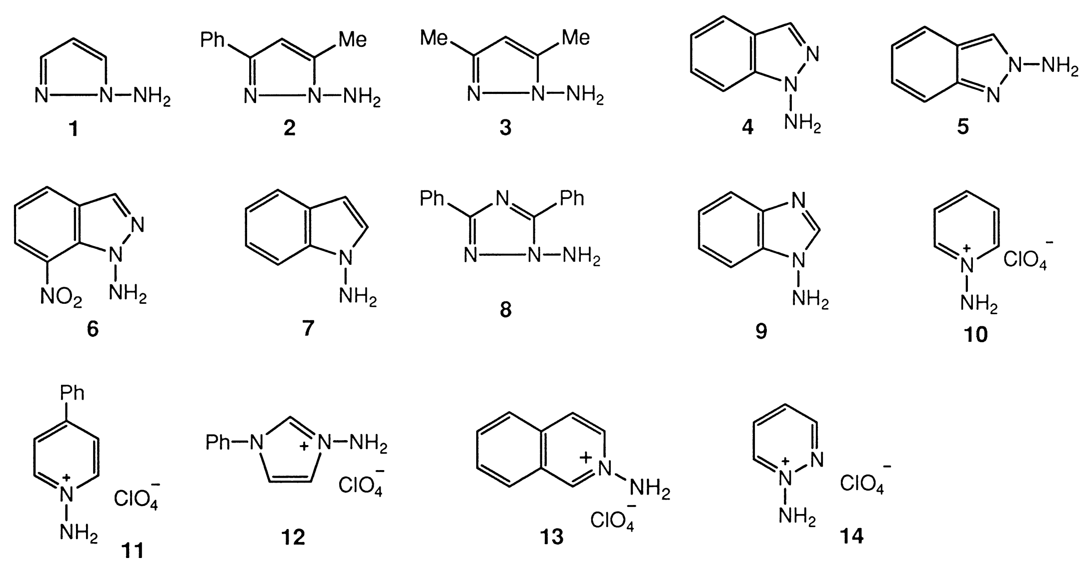
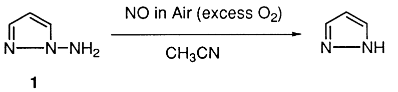


Figure 1. *N*-Aminoazaaromatics used for the reaction.



Scheme 1.

Table 1. The reaction of *N*-aminoazaaromatics with NO in air

N-Aminoazaaromatic	NO (equiv)	Reaction time	Yield (%)[a]
1	1	9 h	65 (33)
	2	5 min	100
2	2	5 min	72 (17)
4	1	5 h	57 (43)
	2	5 min	97
6	2	5 min	92 (6)
10	1	9 h	55 (43)
	2	5 min	90
11	2	5 min	90
12	1	5 h	53 (43)
	2	5 min	100
13	1	5h	51 (19)
	2	5 min	92

[a] The numbers in the parentheses show the recovery (%) of the starting materials.

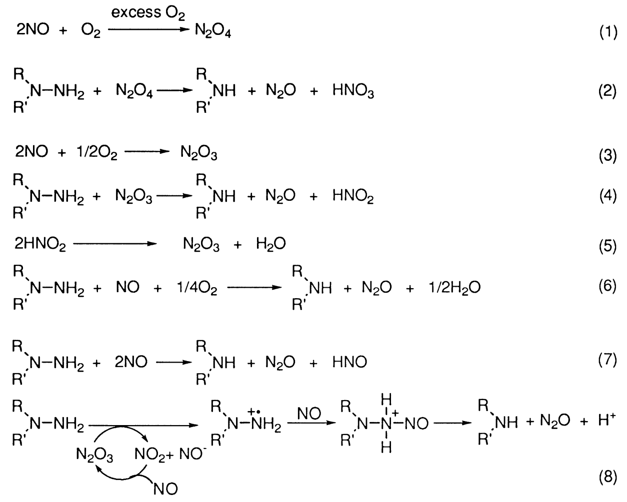
In order to determine the stoichiometry of oxygen, the reaction using *N*-aminopyrazole (**1**) was carried out in the presence of various amounts of NO and O_2, and the results are summarized in Table 2.

When 2 equiv of NO was applied, the reaction completed by the use of 0.25 equiv of O_2. In the case of the use of 0.05 equiv of O_2, the reaction proceeded at nearly half point in the presence of 4 equiv of NO (entry 4). Thus it was suggested that the partial catalytic behavior of O_2 be involved in the reaction. The use of equimolar amount of NO resulted in 80% yield of the deaminated product in the presence of 0.25 equiv of O_2, and excess O_2 caused the slight decrease of the yield (entries 7–10). Thus, there seems to exist at least three pathways that consume different amounts of NO and O_2.

Table 2. The relationship between the yield of the deaminated product and the amounts of NO and O_2 using *N*-aminopyrazole (**1**) as a substrate

Entry	NO (equiv)	O_2 (equiv)	Time (h)	Yield (%)	Recovery (%)
1	2	0.5	1	96	—
2	2	0.25	1	93	—
3	2	0.1	0.5	49	46
4	4	0.05	4	46	52
5	4/3	0.5	1	100	—
6	4/3	0.25	13	73	26
7	1	0.5	1	67	31
8	1	0.5	4	77	20
9	1	0.25	13	80	20
10	1	excess	9	65	33

One of the plausible mechanisms based on the stoichiometry in Table 2 is shown in Scheme 2. In the presence of excess amount of O_2, NO is transformed to NO_2 (or 0.5 N_2O_4), and the reaction might proceed through the electrophilic attack of N_2O_4 to *N*-amino group to give the deaminated product, N_2O, and nitrate ion (eqs (1) and (2)). As decreasing the amount of O_2, the active nitrogen oxide mainly involved might be dinitrogen trioxide N_2O_3 (eq (3)), and N_2O_3 attacks amino group to afford the product, N_2O, and nitrite ion (eq (4)). Nitrite ion thus obtained is known to dimerize to regenerate N_2O_3 (eq (5)), thus the overall stoichiometry of the reaction is thought to be as shown in eq (6). In this occasion, the stoichiometrical amount of O_2 should be 0.25 equiv. Although the two types of the reaction processes mentioned above might consume over 0.25 equiv of O_2, there is another pathway that involves a catalytic contribution of O_2 as shown in Entry 4 of Table 2, in which 0.05 equiv of O_2 afforded 46% of the product. We have also found this type of the O_2 catalytic process in the reaction of aromatic primary amines with NO,[7] and presume that N_2O_3 reacts with the substrate so as to give NO^- and NO_2, which regenerates N_2O_3 via bonding to NO (eqs (7) and (8)).



$$2NO + O_2 \xrightarrow{\text{excess } O_2} N_2O_4 \qquad (1)$$

$$RR'N\text{-}NH_2 + N_2O_4 \longrightarrow RR'NH + N_2O + HNO_3 \qquad (2)$$

$$2NO + 1/2O_2 \longrightarrow N_2O_3 \qquad (3)$$

$$RR'N\text{-}NH_2 + N_2O_3 \longrightarrow RR'NH + N_2O + HNO_2 \qquad (4)$$

$$2HNO_2 \longrightarrow N_2O_3 + H_2O \qquad (5)$$

$$RR'N\text{-}NH_2 + NO + 1/4O_2 \longrightarrow RR'NH + N_2O + 1/2H_2O \qquad (6)$$

$$RR'N\text{-}NH_2 + 2NO \longrightarrow RR'NH + N_2O + HNO \qquad (7)$$

$$RR'N\text{-}NH_2 \xrightarrow[N_2O_3 \;\; NO_2{+}\,NO^-]{} RR'N\text{-}\overset{+\bullet}{N}H_2 \xrightarrow{NO} RR'N\text{-}\overset{H}{\underset{H}{\overset{|+}{N}}}\text{-}NO \longrightarrow RR'NH + N_2O + H^+ \qquad (8)$$

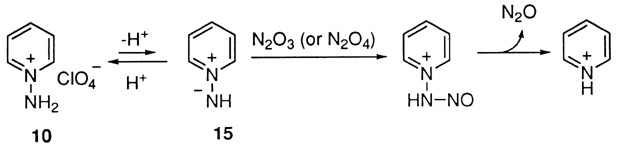
Scheme 2.

The same stoichiometry was observed when *N*-aminopyridinium perchlorate was adopted as a substrate using various amounts of NO and O_2 (Table 3). Consequently, it was suggested that the two types of the substrates undergo the deamination through the same reaction process. Although it seems improbable for *N*-amino quaternary salt to receive electrophilic attack of nitrosating agent, one possible explanation is that *N*-amino quaternary salts initialize the reaction by deprotonation of *N*-amino group to form an imine **15**, which undergoes the reaction with N_2O_3 or N_2O_4 to give an *N*-nitroso derivative (Scheme 3).

The above data indicated that the *N*-aminoazaaromatics react with NO (in the presence of O_2), N_2O_3, and N_2O_4 in different manners, and eliminate these species as a form of N_2O. For studying the applicability of the reaction, we investigated the solvent effect of the reaction, and Table 4a and b show the data. While the reaction proceeded smoothly in any type of solvents in the case of a pyrazole derivative **2**, a pyridinium salt **11** reacted slowly in THF, and gave no deaminated product in ethanol. These data suggest that there can be different reaction paths in these two types of substrates. In ethanol, nitrosating agents would react with the solvent to form a nitrite ester, which could have different reactivity toward **2** and **11**.

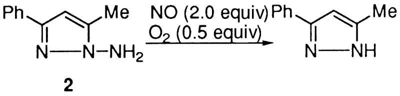
Table 3. The relationship between the yield of the deaminated product and the amounts of NO and O_2 using *N*-aminopyridinium perchlorate (**10**) as a substrate

Entry	NO (equiv)	O_2 (equiv)	Time (h)	Yield (%)	Recovery (%)
1	2	0.5	0.5	100	—
2	2	0.25	1	100	—
3	2	0.1	1	46	50
4	4/3	0.5	4	100	—
5	1	0.5	4	68	30
6	1	excess	9	55	43



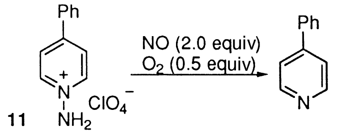
Scheme 3.

Table 4. **a.** The reaction of **2** with NO and O_2 in various solvents



Solvent	Time (h)	Yield (%)	Recovery (%)
CH_2Cl_2	2	92	—
DCE[a]	2	94	—
THF	2	91	—
EtOH	24	82	17
DMF	2	88	2

b. The reaction of **11** with NO and O_2 in various solvents



Solvent	Time (h)	Yield (%)	Recovery (%)
CH_2Cl_2	2	98	—
DCE[a]	2	98	—
THF	2	36	36
EtOH	2	—	90
DMF	2	64	18

[a] DCE, 1,2-dichloroethane.

Table 5 represents the results of deamination reaction of *N*-aminoazaaromatics in water. Except the substrates insoluble in water (**2** and **4**), the reaction proceeded smoothly to give the deaminated product.

Reaction of *N*-aminoazoles with potassium superoxide

Next, we investigated the reaction of *N*-aminoazaaromatics with superoxide. Since superoxide has been known to be a precursor of other active oxygen species[16] and peroxynitrite,[14,15,18] the scavenging of this anion radical is one of the most important issues in the biological system.

Table 5. Deamination of *N*-aminoazzaaromatics with NO (2 equiv) and O_2 (0.5 equiv) in water

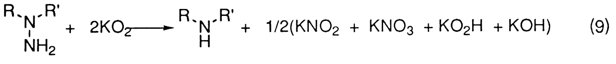
Substrate	Reaction time (h)	Yield (%)
1	3	96
2	2	49
3	2	95
4	1	63
5	1	100
10	3	83
11	1	69
12	1	95
14	1	76

When 1-amino-5-methyl-3-phenylpyrazole (**2**) was allowed to react with potassium superoxide in acetonitrile, the deaminated product was obtained in a good yield, which was measured with ^{1}H NMR using mesitylene as an internal standard. Table 6 shows the results using various amounts of superoxide and 18-Crown-6 (Scheme 4).

The data indicated that the presence of 18-Crown-6 was crucial for the rapid progress of the reaction (entries 1–5), and the reaction demanded 2 equiv of superoxide (entries 5, 8, and 9). Unexpectedly, the presence of O_2 did not afford any influences upon the yields (entries 6, 8, and 5 versus 10, 11, and 12), which indicated that O_2 did not participate in rate determining step(s). The reaction also proceeded by the use of electrogenerated superoxide.

For the understanding of the stoichiometrical relationship of the reaction, the fate of the nitrogen atom of *N*-amino group was investigated, and it was found that nearly half equiv of nitrite and nitrate ion were released in every case (Scheme 5). The amounts of nitrite[19] and nitrate[20] were determined using reported methods.

Another possible nitrogen derivatives such as N_2, N_2O, and NO were not detected in the mixture. Thus, the reaction equation should be formulated as follows (eq (9)).



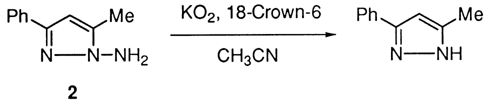
$$RR'N{-}NH_2 + 2KO_2 \longrightarrow RR'NH + 1/2(KNO_2 + KNO_3 + KO_2H + KOH) \quad (9)$$

The other substrates were also applied to the reaction system and the results are summarized in Table 7. Any *N*-aminoazoles tested were found to be good substrates for the reaction, and the stoichiometry of the reaction was in accord with that of eq (9) in every case. The *N*-aminated quaternary salts of azaaromatics, however, resulted in low yields of deaminated products accompanied by complex mixtures.

Table 6. The relationship between the yield of the deaminated product and the amounts of KO_2 using 1-amino-5-methyl-3-phenylpyrazole (**2**) as a substrate

Entry[a]	KO_2 (equiv)	18-Crown-6 (equiv)	Time (h)	Yield (%)	Recovery (%)
1	2	0	25	53	20
2	2	0.5	25	76	4
3	2	2	0.1	67	28
4	2	2	0.2	86	8
5	2	2	1	91	1
6	1	1	0.5	55	41
7	1	1	1	61	35
8	1	1	17	63	31
9	1.5	1.5	3	81	12
10	1	1	0.5	54	44
11	1	1	17	62	32
12	2	2	1	92	1

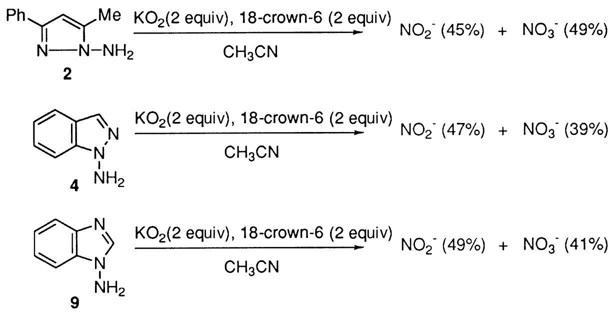
[a] Entries 1–9 were the experiments under argon, and 10–12 were under O_2.



2

Scheme 4.

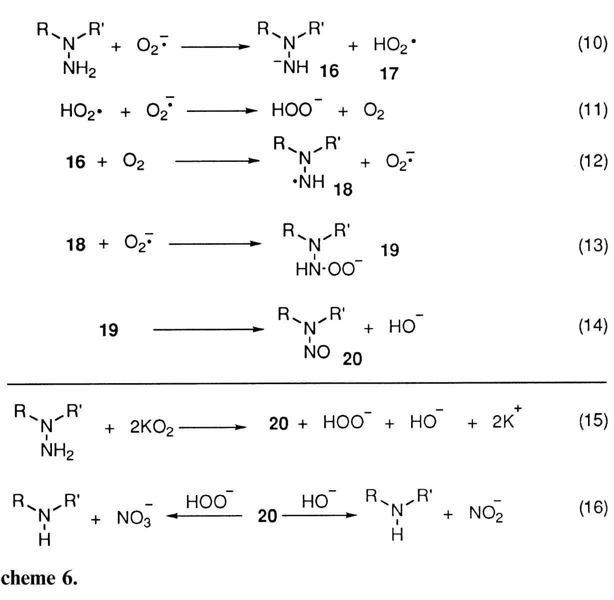
One of the plausible mechanisms is as follows (Scheme 6). The reaction is commenced with abstraction of a proton from *N*-amino group by superoxide to form an *N*-imino anion **16**[21] and hydroperoxy radical **17**. The obtained **17** is well known to react rapidly with superoxide to give hydrogen peroxide and oxygen.[22] Oxygen thus formed might react with **16** to give an aminyl radical **18**, which further reacts with superoxide to afford a peroxy anion **19**. The anion **19** is supposed to decompose to an *N*-nitroso compound **20** and OH^-. The overall stoichiometry of eq (10) to eq (14) is representated as eq (15), which means that the same amount of HOO^- and HO^- was formed by the reactions. The *N*-nitroso compound **20** derived from azaaromatics is thought to be unstable under the conditions to decompose by nucleophilic



Scheme 5.

Table 7. The yield of deaminated product by the reaction of *N*-aminoazaaromatics with potassium superoxide (2 equiv) under Ar

Substrate	Reaction Time (h)	Yield (%)
1	1	82
2	1	91
3	1	80
4	1	84
7	4	75
8	1	97
9	2	91
10	2	13



Scheme 6.

attack of either OH^- or HOO^- to give nitrite or nitrate ion, respectively. In fact, there are few reports concerning stable *N*-nitrosoazoles other than indoles[23] and carbazole.[24] *N*-Nitrosocarbazole was synthesized according to the reported method,[24] and was subjected to the reaction with OH^- or HOO^-. The results showed that denitrosation proceeded at similar rates with both nucleophiles. Thus, in the reaction, these two nucleophilic attacks seem to occur at about the same rate to afford two anions in nearly the same yields. Another possible mechanism for the formation of 1:1 of nitrite: nitrate is hydration of N_2O_4.[25] However, in the presence of equimolar *N*-aminoazole and H_2O, N_2O_4 was found to react quite faster with *N*-aminoazole than H_2O. Thus, we concluded that N_2O_4 (NO_2) was not released in the reaction. In the case of *N*-aminated quaternary salts, *N*-imine **16** is readily formed[13] and would be more stable than those of *N*-aminoazoles, thus the step shown in eq (12) should be slow.

Conclusion

In this paper, we described the deamination of *N*-aminoazaaromatics with various nitrogen oxides. It was found that the substrates reacted rapidly with nitrogen oxides to form N_2O. The reaction proceeded in the presence of various amounts of O_2, and the products were only deaminated azaaromatics and N_2O. In addition, diverse *N*-aminoazaaromatics were found to be applicable for the reaction, and a variety of solvents are available to the reaction including water. This nitrosation–deamination of *N*-amino group has an advantage over that of *C*-amino compounds in that deamination produces a stable azaaromatics whereas *C*-amino derivatives give carbocations or carbon radicals,[7] which might be toxic to the body. In addition, deamination of *N*-aminoazaaromatics with nitrogen oxides is superior to that of general *N*-amino compounds because the latter gives *N*-nitroso compounds, which may be carcinogenic,[26] as final products. Thus the reaction system is thought to be of use for the quenching nitrogen oxides. Moreover, there are many azaaromatics that act as inhibitors of nitric oxide synthase (NOS), some of which involve 7-nitroindazole[27] and 1-phenylimidazole.[28] Our reaction system can afford these compounds by the deamination of corresponding *N*-amino derivatives, and this indicates the reaction might provide a system in which NOS inhibitor is released only in the presence of enough NO.

Moreover, it was found that *N*-aminoazoles are good scavenger of superoxide, which is a precursor of various active oxygens and peroxynitrite. Therefore, they are found to be effective toward most radicals originating from the biological systems. The detailed reaction mechanisms and the application to the biological system is now under investigation.

Experimental

Melting points were measured with a Büchi535 micromelting point apparatus and are uncorrected. The mass spectra were recorded on a JMS-SX102A instrument. The nuclear magnetic resonance spectra (NMR) were measured with JEOL GX400 and LA500 spectrometers using tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; dt, double triplet; dq, double quartet; t, triplet; q, quartet; quin, quintet; m, multiplet. High performance liquid chromatography (HPLC) analysis was carried out using a JASCO UV-1575 PU-1570 system.

Materials. Nitric oxide gas (99.9%) was purchased from Takachiho Chemical Company Ltd, and was passed through 10 M NaOH aqueous solution and a column of 4–8 mesh soda lime to remove NO_X impurities. All chemicals except *N*-aminoazaaromatics were of analytical grade and were used as received.

Synthesis of *N*-aminoazaaromatics

Method A. According to the reported method,[17,29,30] an azaaromatic was dissolved in aqueous NaOH. Hydroxylamine-*O*-sulfonic acid (HAS) in limited amounts was added to the solution, which was kept at 50 °C. Then the mixture was allowed to stir at room temperature to complete the reaction. The mixture was extracted with CH_2Cl_2, and the organic layer was dried over $MgSO_4$, and evaporated off to leave the residue, which was chromatographed on alumina (aluminium oxide 90 (70–230 mesh, Merck)) or silica gel (Kieselgel 60 (70–230 mesh, Merck)) to give the product.

Method B. *O*-Mesitylenesulfonylhydroxylamine (MSH) was synthesized according to the reported method.[31] The activity of MSH, which included considerable amount of water in synthetic process, was determined by iodide–thiosulfate titration, and 2.5 mmol of MSH was dissolved in CH_2Cl_2. Water was separated off, and the organic solution was dried over $MgSO_4$. An azaaromatic (2.0 mmol) and NaH (2.5 mmol) were reacted in THF at 0 °C for 30 min, then the MSH solution was added dropwise. After the mixture was allowed to stand at room temperature for several hours, methanol was added to quench excess NaH, and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on alumina or silica gel to give the product. Some of the *N*-aminoazaaromatics were difficult to separate from the starting materials by simple chromatography. In these cases, *N*-aminoazaaromatics were transformed to imines by the treatment with acetone in the presence of a catalytic amount of *p*-toluenesulfonate. After the separation of the imines and the starting material, the isolated imines were hydrolyzed to give the *N*-amino compounds. Among 14 *N*-aminoazaaromatics used for the reaction,[17,32–34] **6**, **8**, **11**, and **12** are new compounds.

1-Amino-7-nitroindazole (6). This was synthesized using method B. Yellow powder from hexane–ethyl acetate: mp 157–158 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 6.49 (2H, bs), 7.26 (1H, dd, $J = 8.0$ Hz, 7.6 Hz), 7.95 (1H, d, $J = 7.6$ Hz), 8.08 (1H, d, $J = 8.0$ Hz), 8.21 (1H, s). Anal.

calcd for $C_7H_6N_4O_2$: C, 47.19; H, 3.39; N, 31.45. Found: C, 47.43; H, 2.81; N, 31.15.

1-Amino-3,5-diphenyl-1,2,4-triazole (8). This was synthesized using method B. Colorless granules from ethanol: mp 193–194 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39–7.52 (6H, m), 8.12 (2H, dd, *J* = 6.6Hz, 2.0 Hz), 8.25 (2H, dd, *J* = 7.3Hz, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 126.07, 127.06, 128.30, 128.47, 128.78, 129.20, 129.98, 130.65, 152.47, 158.31. Anal. calcd for $C_{14}H_{12}N_4$: C, 71.17; H, 5.12; N, 23.71. Found: C, 71.04; H, 4.97; N, 23.69.

1-Amino-4-phenylpyridinium perchlorate (11). This was synthesized using method A. Pale yellow powder from ethanol: mp 120–121 °C; ^1H NMR (400 MHz, CD_3OD) δ 7.60–7.62 (3H, m), 7.92–7.94 (2H, m), 8.29 (2H, d, *J* = 7.0 Hz), 8.73 (2H, d, *J* = 7.0 Hz). Anal. calcd for $C_{11}H_{11}ClN_2O_4$: C, 48.80; H, 4.10; N, 10.35. Found: C, 49.08; H, 3.88; N, 10.26.

1-Amino-3-phenylimidazolium perchlorate (12). This was synthesized using method B. Colorless plates from ethanol: mp 132–133 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.49 (5H, s), 7.53 (1H, d, *J* = 1.5 Hz), 7.69 (1H, d, *J* = 1.5 Hz), 9.11 (1H, s). Anal. calcd for $C_9H_{10}ClN_3O_4$: C, 41.63; H, 3.88; N, 16.18. Found: C, 41.76; H, 4.18; N, 15.97.

General procedure for the reaction of *N*-aminoazaaromatics with nitric oxide in the presence of oxygen. The substrate (0.2 mmol) was placed in a two-necked flask equipped with a rubber septum and a three-way stopcock, one outlet of which was attached to an Ar balloon, and another to a pump. The flask was degassed in vacuo and filled with Ar gas. A solvent (10 mL) was added and the solution was bubbled with Ar gas for 20 min, then the flask was sealed. NO gas was passed through a column of soda lime and a necessary amount was measured using a Hamilton gas-tight syringe, and added to the reaction vessel. Then, a certain amount of oxygen was added and the reaction mixture was allowed to stir at room temperature. When the reaction was completed, Ar was bubbled to degass excess NO and O_2, and the solvent was evaporated off to leave the residue, whose analysis was performed by ^1H NMR using mesitylene or 1,4-dioxane as an internal standard. In the cases that the products are volatile, the reaction was carried out in deuterated solvents, and the mixture was analyzed directly by NMR.

The analysis of gaseous products. The gaseous products were analyzed by Shimadzu GC-14BPTF gas chromatography with TCD detector using SHINCARBON T (N_2O) and Porapak Q (N_2 and O_2) columns. Since N_2O was found to partially dissolve in CH_3CN (ca. 25%) or H_2O (ca. 10%) under the reaction conditions, the analyzed data were corrected based on these results. When the compound **2** (0.2 mmol) reacted with NO (0.4 mmol) and O_2 (0.1 mmol), the analysis of the gaseous part showed that N_2O was obtained in 70% (in CH_3CN) or 85% (in H_2O). Thus, it was concluded that N_2O was formed stoichiometrically. In the case of the compound **11** in H_2O, the analysis indicated that 90% of N_2O was present in the gas phase. This datum also suggested that an equivalent of N_2O was formed in the reaction.

General procedure for the reaction of *N*-aminoazaaromatics with potassium superoxide. KO_2 and 18-Crown-6 was dissolved in CH_3CN, and the solution was bubbled with dry Ar gas for 20 min when necessary. Then the flask was sealed, and a substrate in CH_3CN was introduced to the reaction mixture using a syringe. The mixture was allowed to stir at room temperature for the desired hours. Thereafter, the solvent was evaporated off to leave the residue, whose analysis was performed by ^1H NMR using mesitylene or 1,4-dioxane as an internal standard. In the cases that the products are volatile, the reaction was carried out in CD_3CN, and the mixture was analyzed directly by NMR. Electrogenerated superoxide also afforded a similar result. In the experiment, *N*-aminopyrazole was dissolved in 40 mL of 0.1 M tetraethylammonium perchlorate solution of acetonitrile and a stream of oxygen was bubbled into the solution through a gas dispersion tube which was inserted into the cathode chamber of a H cell containing platinum electrode. The electroreduction of oxygen was carried out with Nikko Keisoku potentiogalvanostat NPGS-2501. The potential was set and maintained at −1.10 V versus SCE until the starting material was disappeared.

The analysis of HNO_2 and HNO_3. The amount of nitrite ion was determined by Saltzman reagent method.[19] HNO_3 was determined using the reported method which included brucine as a reagent.[20]

Reaction of *N*-nitrosocarbazole. *N*-Nitrosocarbazole was synthesized according to the reported method.[24] It (0.05 mmol) was dissolved in CH_3CN (1 mL), and the solution was bubbled with dry Ar gas for 15 min, then the flask was sealed. 30% H_2O_2 (0.5 mmol) was added to the solution, and the mixture was allowed to react for 2 h to give a quantitative yield of carbazole. The same result was obtained when *N*-nitrosocarbazole (0.1 mmol) was subjected to the reaction with KOH powder (2 mmol), 18-Crown-6 (2 mmol), and H_2O (2 mmol) in CH_3CN (2 mL) for 2 h.

The experiment for excluding NO_2 as a donor of nitrite and nitrate ion. KOH (1.2 mmol) was suspended in CH_3CN, and the solution was bubbled with dry Ar gas for 15 min. Then the flask was sealed, and 1-amino-5-methyl-3-phenylpyrazole (**2**) (0.6 mmol) in CH_3CN (1 mL) was introduced to the reaction mixture using a syringe. Then NO_2 (0.6 mmol) was added with a gas-tight syringe, and the mixture was allowed to stir at room temperature for 2 h. Thereafter, Ar was bubbled to degass excess NO_2, and the solvent was separated to two parts, and one was evaporated off to leave the residue, whose analysis was performed by ^1H NMR. The result showed that the deaminated product was obtained in 40% yield accompanied by the recovery of 60% of **2**. The other part was subjected to the analysis of nitrite and nitrate ion, and it was shown that 0.3 mmol of nitrate ion was formed accompanied by no nitrite ion.

These data indicated that NO_2 reacted with **2** much faster than with H_2O, thus the formation of 1:1 of nitrite and nitrate in the reaction of *N*-aminoazoles and superoxide was not derived from hydrolysis of NO_2.

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