Reactions of indoles with nitrogen dioxide and nitrous acid in an aprotic solvent

Paola Astolfi,[a] Maria Panagiotaki,[a] Corrado Rizzoli[b] and Lucedio Greci*[a]

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The reaction of 2-phenyl- and 1-methyl-2-phenylindole with nitrogen dioxide or with nitrous acid ($NaNO_2$–CH_3COOH) in benzene leads mainly to the formation of the isonitroso and 3-nitroso indole derivatives, respectively. When reacted with nitrous acid, 1-methyl-2-phenylindole gives also the corresponding azo-bis-indole in good yields. The reaction of indole with nitrogen dioxide leads to 2-(indol-3-yl)-3*H*-indol-3-one as the main product together with small amounts of 2-(indol-3-yl)-3*H*-indol-3-oxime; whereas the major product obtained when the same indole is reacted with nitrous acid is represented by 2-(indol-3-yl)-3*H*-indol-3-oxime. The reaction of 3-alkyl substituted indoles with nitrogen dioxide is rather complex and results in the formation of different nitro indoles, whereas nitrosation is observed when nitrous acid is used. Crystal structures of 2-(indol-3-yl)-3*H*-indol-3-one and of 4-nitro-*N*-acetyltryptamine have been determined by X-ray analysis.

Introduction

Nitrogen monoxide (•NO) is an important physiological messenger involved in many *in vivo* processes including neurotransmission, immune regulation, smooth muscle relaxation and platelet inhibition.[1] But, simultaneous to these physiologically important pathways is the formation of reactive species that may result in cytotoxic and mutagenic events. For example, from the reaction between •NO and superoxide ($O_2^{\bullet -}$), peroxynitrite anion (–OONO) is formed, which is responsible for the oxidation of many types of molecules such as sulfhydryls,[2] methionine,[3] ascorbate,[4] DNA[5] and lipids.[6] Moreover, peroxynitrite may promote nitration of aromatic molecules such as tyrosine.[7] Peroxynitrite is not the sole reactive species with deleterious effects derived from •NO. Dinitrogen trioxide (N_2O_3) formed from •NO in the presence of oxygen readily attacks thiols[8] and amines[9] leading to S-nitrosothiols and N-nitrosamines, respectively. Autooxidation of •NO results also in the formation of nitrogen dioxide ($^{\bullet}NO_2$), although the main pathways responsible for its formation are the homolytic decomposition of peroxynitrous acid (HOONO),[10] the reaction of peroxynitrite with CO_2[11] and the peroxidase-catalyzed oxidation of nitrite.[12] Since this molecule is particularly soluble in apolar environments, such as cell membranes and hydrophobic protein domains, the pathophysiological consequences[13] of low levels of endogenous nitrogen dioxide are currently under investigation. It is a very reactive species which can react through different pathways: H-atom abstraction,[14] electron transfer reaction,[15] addition to unsaturated bonds[16] and recombination with other radicals.[17] This complex reactivity was pointed out in a previous study[18] by us on the interaction between nitrogen dioxide and various alkyl-substituted phenols. In particular, it was found that many factors, such as the bond dissociation enthalpies (BDE), the oxidation potentials, the solvent used, together with the nature of the substrate, had to be considered in order to explain the specific reaction pathway followed and the products obtained. In order to gain further insights on the reactivity of nitrogen dioxide, we extended this study to other organic compounds such as indoles, whose structure is very common among biologically important molecules. In fact, tryptophan, melatonin and serotonin are all indolic compounds as well as pindolol,[19] a drug with a potential scavenging effect on •NO, –OONO and $O_2^{\bullet -}$. Therefore, the reaction between indoles **1–3** (Chart 1) with gaseous nitrogen dioxide was studied. Furthermore, the reactivity of the same indoles with nitrous acid HNO_2 was also considered because it is the ultimate oxidative species derived from •NO (eqn 1–5) and could perhaps be generated in acidic cell compartments.

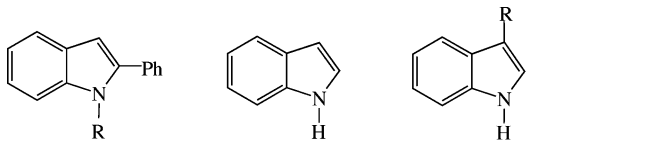
$$^{\bullet}NO + O_2 \rightarrow ONOO^{\bullet} \quad (1)$$

$$ONOO^{\bullet} + {}^{\bullet}NO \rightleftarrows ONOONO \quad (2)$$

$$ONOONO \rightarrow 2\,{}^{\bullet}NO_2 \quad (3)$$

$$^{\bullet}NO + {}^{\bullet}NO_2 \rightarrow N_2O_3 \quad (4)$$

$$N_2O_3 + H_2O \rightarrow 2\,HNO_2 \quad (5)$$



1 a: R = H
b: R = Me

2

3 a: R = Me
b: R = $(CH_2)_2NHCOMe$
c: R = $(CH_2)_2NHCOPh$

Chart 1

[a]*Dipartimento di Scienze dei Materiali e della Terra, Università Politecnica delle Marche, via Brecce Bianche, I-60131, Ancona, Italy. E-mail: l.greci@univpm.it; Fax: +39-071-2204714; Tel: +39-071-2204408*
[b]*Dipartimento di Chimica Generale, Chimica Analitica, Chimica Fisica, Università di Parma, Viale G. P. Usberti, I-43100, Parma, Italy*

Results and discussion

Indoles **1–3** were reacted at room temperature with gaseous nitrogen dioxide in 1 : 1 and 1 : 2 ratios or with nitrous acid, generated *in situ* from the reaction between sodium nitrite and acetic acid. In all cases, benzene was used as the solvent. All the products obtained were identified on the basis of their spectroscopic data or by comparison with authentic samples; for compounds **8** and **20b** X-ray analysis was also performed.

Reaction of indoles 1a, 1b and 2

When indoles **1a** and **1b** (2-substituted indoles) and indole **2** were reacted in benzene with $^{\bullet}NO_2$ (or N_2O_4) in a stoichiometric ratio or in a 1 : 2 ratio and with nitrous acid, generated from sodium nitrite and acetic acid, compounds reported in Scheme 1 were obtained.

Isolated products, together with the corresponding yields, are reported in Table 1.

1-Methyl-2-phenyl-3-nitroso indole **4** and 2-phenyl-3-oxime indole **5** (the tautomeric form of 2-phenyl-3-nitroso indole) most likely derive from an electrophilic attack of the specific nitrosating agent on the nucleophilic position (C-3)[20] of the indole nucleus. A nitroso indole is thus formed, but if the nuclear nitrogen is unsubstituted, as in the case of indole **1a**, it readily tautomerizes to the isomeric 3-oxime product **5**. When nitrogen dioxide was used, the nitrosating species was represented by dinitrogen tetraoxide N_2O_4. In fact, nitrogen dioxide may exist as a monomer with the unpaired electron delocalized throughout the molecule or as a dimer, whose structures are shown in Fig. 1.

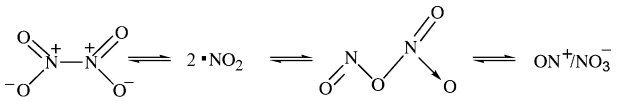
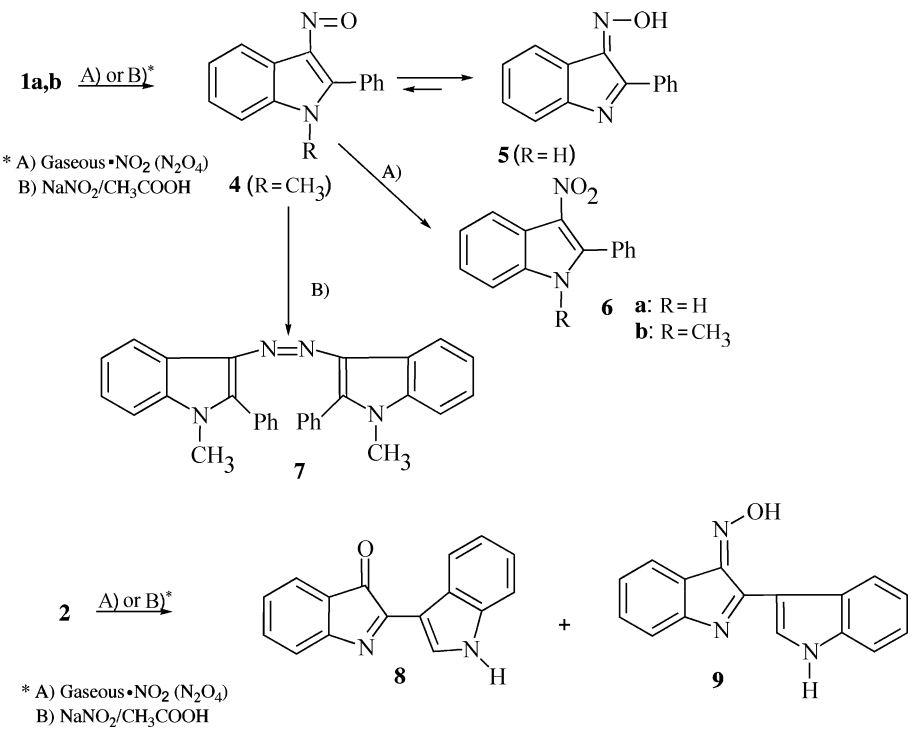


Fig. 1 Possible chemical structures of N_2O_4 (the dimeric form of $^{\bullet}NO_2$).

Structures **C** and, above all (at least in our experimental conditions), **B**[21] account for the nitrosating ability of dinitrogen tetraoxide as demonstrated by its efficient reaction with thiols resulting in S-nitrosothiols.[22] Other nucleophiles, such as phenols,[18] amines[23] or, as in the present case, indoles react with the unsymmetrical tautomer of N_2O_4 ($ON–NO_3$) to form nitrosated products.

If an excess of nitrogen dioxide was used, the reactions of indoles **1a** and **1b** were very fast (in 10 minutes the starting indole was completely consumed) and nitroso (or isonitroso) indoles **5** and **4**



Scheme 1

Table 1 Products obtained in the reactions of indoles **1a**, **1b** and **2** with nitrogen dioxide and nitrous acid with the corresponding percentage yields (in brackets)

	Products obtained in:		
Indoles	Gaseous $^{\bullet}NO_2$ 1 : 1	Gaseous $^{\bullet}NO_2$ 1 : 2	HNO_2
1a	**1a** (15) **5** (85) 2 hrs	**5** (70) **6a** (30) 10 mins	**5** (90) 4 hrs
1b	**1b** (20) **4** (80) 2 hrs	**4** (30) **6b** (70) 10 mins	**1b** (33) **4** (32) 7 (35) 4 hrs
2	**8** (80) **9** (20) 10 mins		**8** (20) **9** (80) 2 hrs

were partially oxidized to the corresponding 3-nitro indoles **6a** and **6b** (Table 1). In order to check whether formation of 3-nitro indole **6b** (or **6a**) was a consequence of oxidation of 3-nitroso indole **4** (or **5**), we separately treated the latter derivative with $^{\bullet}NO_2$. TLC analysis showed that compound **6b** (or **6a**) was actually formed; this indicates that 3-nitro indoles derive from the corresponding 3-nitroso derivatives and not from a direct nitration.

Several nitrosating agents could be active when nitrous acid was used (eqn 6–10), although in our experimental conditions (addition of an excess of acetic acid to sodium nitrite in an aprotic solvent and in the absence of oxygen) the electrophilic attack could be likely due to nitrosyl acetate CH_3COONO (eqn 9), a powerful nitrosating agent.[24]

As reported in Scheme 1 and Table 1, the reaction of indole **1b** with $NaNO_2$–CH_3COOH was characterized by the formation of nitroso indole **4** and, in good yield, of azo-bis-indole **7**. The mechanism which leads to the formation of this latter compound (Scheme 2) is very similar to that described previously by us for the reaction between **1b** and nitrogen monoxide.[25] Nitroso compounds have well-documented spin trapping abilities[26,27] and compound **4**, as such, may likely trap a $^{\bullet}NO$ molecule generated during nitrous acid decomposition (eqn 10) affording the corresponding *N*-nitroso nitroxide **10**. Coupling with another $^{\bullet}NO$ molecule, followed by rearrangement to nitrate arenediazonium salt **11** and reaction with the starting indole, justifies the formation of compound **7**.

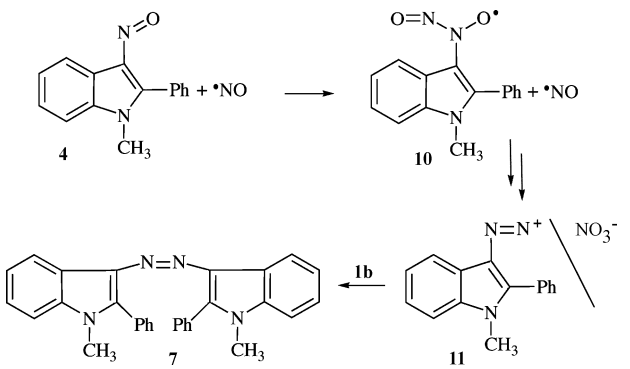
$$CH_3COOH + NaNO_2 \rightarrow HNO_2 + CH_3COONa \quad (6)$$

$$2\ HNO_2 + H^+ \rightleftarrows H_2NO_2^+ \rightleftarrows NO^+ + H_2O \quad (7)$$

$$2\ HNO_2 \rightleftarrows N_2O_3 + H_2O \rightleftarrows {}^{\bullet}NO_2 + {}^{\bullet}NO \quad (8)$$

$$CH_3COOH + HNO_2 \rightleftarrows CH_3COONO + H_2O \quad (9)$$

$$3\ HNO_2 \rightarrow {}^{\bullet}NO + HNO_3 + H_2O \quad (10)$$



Scheme 2

It could be observed that an electron transfer process between indole **1a** (or **1b**) and NO^+ (eqn 7) should be possible, in analogy with what is reported in the literature for the nitrosation of aromatic hydrocarbons with the nitrosonium cation.[28] But, the reaction between indole **1b** and NO^+ has already been studied[29] and the comparison of the products obtained in the two reactions (with HNO_2 and with NO^+) indicates that the mechanisms active in the two cases are different. Moreover, azo-bis-indole **7** was obtained from the reaction with HNO_2 and not from NO^+ or from gaseous $^{\bullet}NO_2$ and this fact further supports the hypothesis that nitrosation by HNO_2 or by N_2O_4 is an electrophilic process and that $^{\bullet}NO$, necessary for the formation of compound **7** observed with nitrous acid, may solely derive from the decomposition of HNO_2 as described in eqn 10. If a radical mechanism was admitted for the reaction between **1b** and $^{\bullet}NO_2$ (N_2O_4), then compound **7** should have been isolated.

When indole **2** was reacted with nitrogen dioxide, oxidation was the main process observed and nitrosation only a minor path. In fact, 2-indolyl-3-one-3*H*-indole **8** was obtained in 80% yield; its crystal structure is reported in Fig. 2. The reaction with nitrous acid afforded the nitrosated product, 2-indolyl-3-oxime-3*H*-indole **9** (Table 1), in almost quantitative yields. Two different mechanisms have to be considered to justify the formation of compounds **8** and **9**: a radical path for the reaction of **2** with $^{\bullet}NO_2$ (which leads to **8**) and an electrophilic attack by CH_3COONO to give compound **9**.

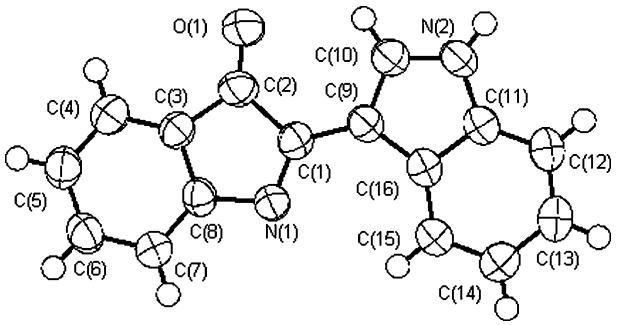
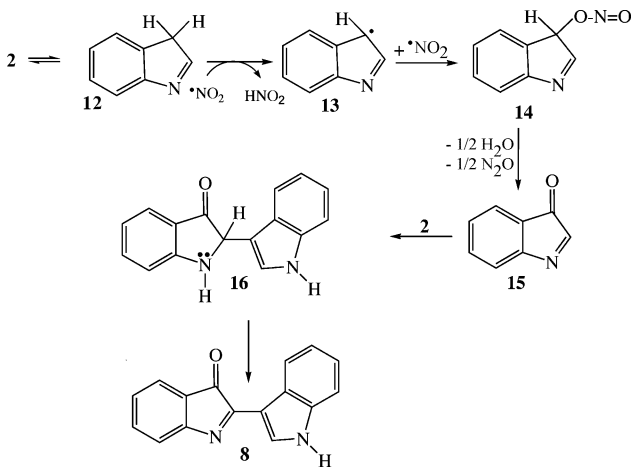


Fig. 2 An ORTEP view of compound **8** (50% probability ellipsoids).

As reported in Scheme 3, indolyl radical **13** may be considered as the key intermediate when the reaction is carried out with gaseous nitrogen dioxide. It may derive from a hydrogen atom abstraction of the C-3 hydrogen of the indolenine tautomer **12**, the calculated BDE value for the H–O bond in nitrous acid[30] being *ca.* 78 kcal mol^{-1} and the BDE for the C–H bond of the sp^3 carbon atom in indolenine **12**[31] being 70 kcal mol^{-1} (hydrogen abstraction from the nitrogen atom in the pyrrole ring would require 88 kcal mol^{-1}).[31] A coupling reaction between **13** and nitrogen dioxide (through



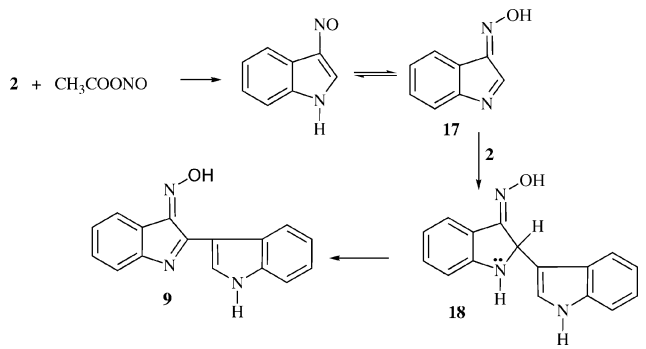
Scheme 3

its oxygen atom) would lead to indol-3-one **15** which was never isolated. In fact, a rapid nucleophilic addition[32] of another indole molecule on this intermediate followed by oxidation[32b] affords compound **8**.

It cannot be excluded that indolyl radical, once formed, may react with molecular oxygen (the reactions with $^{\bullet}NO_2$ were carried out in the presence of O_2) at C-3[33] to form a peroxyl radical which, after dimerization and decomposition, gives the oxidized product **15** and hence **8**. In order to clarify this point, the reaction between indole **2** and $^{\bullet}NO_2$ was repeated using nitrogen dioxide generated by thermal decomposition of $Pb(NO_3)_2$ in the absence of oxygen (data not shown). Indolinone **8** was obtained also in this case and in the same amount as in the presence of oxygen. Thus, it may be concluded that the coupling of indolyl radical with $^{\bullet}NO_2$ is the reaction that actually takes place.

Small amounts of 2-indolyl-3-oxime-3*H*-indole **9** precipitated from the benzene reaction solution together with indolinone **8** when indole **2** was reacted with nitrogen dioxide. This means that electrophilic nitrosation by $^{\bullet}NO_2$ (or its dimer) of indole may take place as described above for the other indoles, although it is clear that coupling of indolyl radical with nitrogen dioxide (through the oxygen atom, as described in Scheme 3) remains the favoured reaction pathway. No differences were observed if different indole–$^{\bullet}NO_2$ ratios were used.

2-Indolyl-3-oxime-3*H*-indole **9** was the product isolated, in very high yields (*ca.* 80%), in the presence of $NaNO_2$–CH_3COOH. Nitrosation (most likely by nitrosyl acetate, as discussed above) was the process occurring also in this case, but indole-3-one oxime **17** (the tautomer of 3-nitrosoindole, the expected product) was never isolated. Similarly to compound **15**, it is a highly reactive species which readily undergoes 1,2-nucleophilic addition of an indole molecule followed by oxidation giving the isonitroso derivative **9** (Scheme 4). In addition to this latter compound, indolinone **8** was also isolated (20%). It was formed, through the mechanism shown in Scheme 3, as a consequence of the presence of nitrogen dioxide in the reaction milieu after the equilibria described in eqn 8.



Scheme 4

From the analysis of the results reported in the present section, it may be observed that nitrosation is the favoured reaction path for indoles **1a**, **1b** and **2** in both reaction conditions. The nitrosating agent is most likely represented by N_2O_4 when the reactions are carried out with gaseous $^{\bullet}NO_2$ and by CH_3COONO using the $NaNO_2$–CH_3COOH system. In both cases, an electrophilic attack of these species on the nucleophilic indole molecules leads to the isolated products. Only in the reaction of indole **2** with $^{\bullet}NO_2$ a different mechanism (a radical one) is active and it likely proceeds through a hydrogen atom transfer step.

Reaction of indoles 3a–c

The reaction of 3-alkyl substituted indoles (3-methylindole **3a**, *N*-acetyltryptamine **3b** and *N*-benzoyltryptamine **3c**) with nitrogen dioxide or nitrous acid was also studied in order to better understand the reactivity of this class of biologically important compounds (tryptophan, melatonin or serotonin are all 3-substituted indoles) towards reactive nitrogen–oxygen species. Unfortunately, the results obtained were not so straightforward as in the cases discussed above. In fact, when indoles **3a–c** were reacted with $^{\bullet}NO_2(N_2O_4)$, complex crude reaction mixtures were obtained in all cases (with the three different indoles used and in both the ratios, 1 : 1 or 1 : 2) and, even after chromatography, only few products were identified. In all cases, the corresponding yields were less than 2%. The identified products are represented by nitro derivatives and it means that nitration occurs even when nitrogen dioxide is used in a stoichiometric ratio and, unlike reactions with indoles **1a** and **1b**, no nitroso derivatives are formed. In fact, when the crude reaction mixtures were examined by ^{1}H NMR spectroscopy, the peaks due to the 1-nitroso derivatives were not observed in the spectra. The isolated nitro compounds are 4- and 6-nitro-3-substituted indoles **20** and **21**. The crystal structure of 4-nitro-*N*-acetyl-tryptamine **20b** is reported in Fig. 3. 1-Nitro-3-substituted indoles **22** are also likely to be formed and 1-nitro-*N*-acetyl-tryptamine **22b** was tentatively identified although the exact structure is yet to be determined. A mass spectrum shows that **22b** is a nitro derivative of *N*-acetyl-tryptamine, confirming the molecular mass ($m/z = 247$, M^+) with the radical $m/z = 201$ [$M–NO_2$]; the ^{1}H NMR spectrum shows approximately the same δ values as the corresponding 1-NO derivative **23b** but only one conformer (see discussion below).

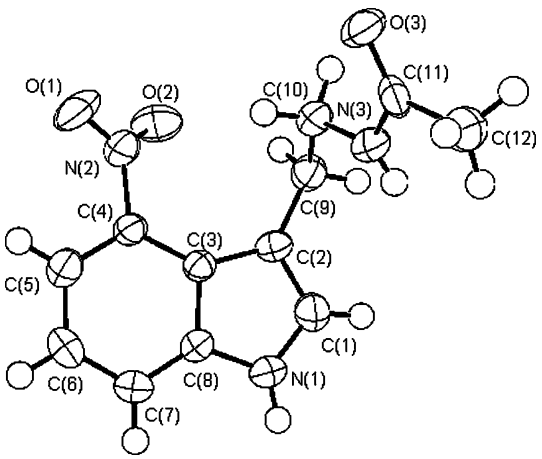
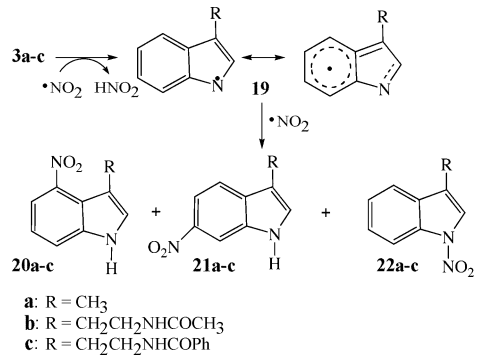


Fig. 3 An ORTEP view of compound **20b** (50% probability ellipsoids).

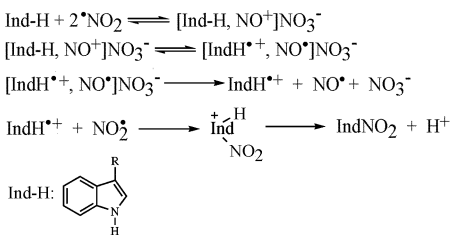
As shown in Scheme 5, a free radical mechanism most likely occurs: an initial hydrogen abstraction from the nitrogen atom of the indole ring by nitrogen dioxide leads to the formation of an indolyl radical **19** in which the unpaired electron may be delocalized throughout the molecule. In analogy with the literature reports for melatonin (another 3-alkyl substituted indole),[34] one may assume that hydrogen atom abstraction is a feasible process



Scheme 5

due to the stabilization of the indolyl radical and in particular of the nitrogen-centred radical, despite the first step being slightly endothermic. The radical intermediate resulting from hydrogen atom abstraction at N1 is, in fact, the most stable of all the possible radicals.[35] Coupling of indolyl radical with another $^{\bullet}NO_2$ molecule leads to the formation of the different nitro indoles identified.

An electron transfer process followed by deprotonation of the radical cation initially formed may be an alternative route to indolyl radical. In fact, nitrogen dioxide is a good hydrogen abstractor and also a good oxidant [$E^{\circ\prime}(^{\bullet}NO_2–NO_2^-) = 0.99$ V *vs.* NHE],[36] thus it may react with indoles **3a–c** (the reduction potentials for the corresponding indolyl radical cations, $E^{\circ\prime}$(indolyl radical, H^+–indole), are *ca.* 1 V)[37] leading to the corresponding indolyl radical cations which, in the reaction medium, are in equilibrium with the corresponding indolyl radicals. One cannot exclude that the mechanism for direct nitration of indoles **3a–c** could be similar to that reported in the literature for aromatic hydrocarbons,[38] which describes the formation of an electron donor–acceptor complex followed by an electron transfer within the complex (Scheme 6). The radical cation thus formed may rapidly homolytically react with $^{\bullet}NO_2$ affording the nitrated products.

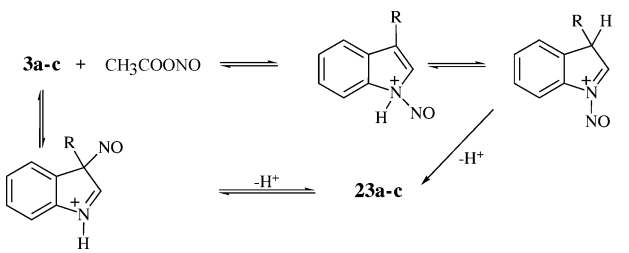


Scheme 6

As already stated, reactions between nitrogen dioxide and indoles **3a–c** were particularly complex: mixtures of different compounds difficult to isolate were obtained. Since the three indoles behaved in the same way, only the reaction of **3b** is reported as a representative of the 3-alkyl indoles.

The results obtained in these reactions are, at least partially, in agreement with the data found in the literature concerning the reaction of biologically relevant indoles, such as tryptophan or melatonin, with reactive nitrogen species, RNS. In fact, nitration is the process observed in all cases, both in the reactions described here as well in the literature reports;[34,39] 1-nitro indoles are formed together with 4- and 6-nitro indoles and, as reported in ref. 39*c*, 1-nitro derivatization is obtained directly from the starting indole. However, the formation of 1-nitroso indoles, due to the nitrosating ability of N_2O_4, was never observed in our experiments, contrary to that reported for the reaction between *N*-acetyl tryptophan or melatonin and nitrogen dioxide described in ref. 39*c* or 43. Noteworthy is the fact that the majority of literature reports on this subject deal with the reaction of these indoles with $^{\bullet}NO$ in the presence of oxygen, and hence in the presence of both $^{\bullet}NO$ and $^{\bullet}NO_2$, or with peroxynitrite in aqueous solutions, which generates $^{\bullet}NO_2$ during its decomposition, but never directly with nitrogen dioxide. This may be the reason for the different results obtained in the present work. In fact, if $^{\bullet}NO$ and $^{\bullet}NO_2$ are simultaneously present in the same reaction milieu, the formation of N_2O_3 (eqn 4), a nitrosating agent, has to be taken into account and hence it is likely that nitroso indoles may derive from the action of N_2O_3 instead of $^{\bullet}NO_2$. When peroxynitrite is used, nitrosation of melatonin[39*a*,40] and of secondary amines[41] easily occurs in aqueous solution especially at alkaline pH, reaction conditions completely different from those used in the present study. In addition, several reactive species are formed during the decomposition of peroxynitrite ($OH^{\bullet}$, $CO_3^{\bullet-}$, *etc.*) and this fact may justify the formation of oxidation products, as described for melatonin.[40,42]

N-Nitroso indoles **23a–c** were the sole products isolated when **3a–c** were reacted with $NaNO_2$–CH_3COOH: they were obtained in 80% yield together with 20% of starting indole (even after 24 hours the reactions did not go to completion). As described above for the reaction of indoles **1** or **2** in the same conditions, nitrosation derives from the electrophilic attack of the nitrosating agent (most likely of nitrosyl acetate) on the nucleophilic centre of the indole molecule. In particular, also in this case the electrophile may attack the carbon atom in position 3, although a substituent is already attached to it, followed by an internal rearrangement of the NO group and, in particular, a 1–3 rearrangement, leading to the formation of *N*-nitroso indoles, although direct attack on the nitrogen atom is also possible (Scheme 7).[43] The presence of the *N*-nitroso group in compounds **23a–c** was clearly evident in their ^{1}H NMR spectra which show, in the aliphatic region, double signals for the methyl groups of **23a** and **23b** and complex multiplets for the methylene groups of **23b** and **23c**, deriving from the overlapping of different signals. When the same spectra were recorded with a 600 MHz spectrometer, it was possible to clearly distinguish the different signals. For example, the ^{1}H NMR spectrum of compound **23b** shows two different singlets for the methyl group at $\delta = 1.97$ and 2.00 in a 1 : 3 ratio and two triplets and two quartets for each of the methylene groups, always in the same ratio. These finding are in agreement with the possibility of two different conformers, *Z* and *E*, generated by the *N*-nitroso group.[44]



Scheme 7

The assignment of the protons in the aromatic region is more difficult, since all the peaks are composite peaks deriving from the overlapping of two different signals. The three compounds (**23a–c**) show a very similar pattern for the peaks in the region between $\delta = 8.00$ and 8.50 ppm corresponding not to a proton but only to a fraction of it. Furthermore, if the two multiplets assigned to H-7 were integrated, the sum of their integration corresponded not to a proton but to half of it. Besides the ^{1}H NMR spectra, these compounds also have characteristic mass spectra. In fact, in all cases, the peak with the highest mass value did not correspond to the molecular ion but to a dimer obtained from the dimerization of the indolyl radical formed after cleavage of the N–NO bond during fragmentation.

Molecular geometry of 2-(indol-3-yl)-3*H*-indol-3-one (8) and of 4-nitro-*N*-acetyltryptamine (20b)

The molecular structures of compounds **8** and **20b** are shown in Fig. 2 and 3, respectively. For both compounds, bond lengths and angles are within normal ranges.[45] In compound **8**, the value of the N(1)=C(1) bond length (1.310(5) Å) is consistent with the localization of the double bond. The indole rings are nearly coplanar, the dihedral angle formed by the mean planes through them being 5.87(2)°. In this conformation, a weak C–H···O intramolecular hydrogen interaction is observed (C(10)···O(1), 2.965(6) Å; H(10)···O(1), 2.39 Å; C(10)–H(10)···O(1), 120°). In the crystal packing, the molecules are linked by N–H···N hydrogen bonds (N(2)···N(1)′, 3.134(4) Å; H(2)···N(1)′, 2.29 Å; N(2)–H(2)···N(1)′, 166°. Symmetry code: $' = -x, -y, -1/2 + z$), to form zigzag chains running parallel to the *c* axis.

In compound **20b**, the C(9)/C(10)/N(3)/C(11)/O(3)/C(12) atoms of the acetaminoethyl chain lie in a plane (maximum deviation from the planarity of 0.079(4) Å for N(3)), which forms a dihedral angle of 80.05(7)° with the mean plane through the indole ring. The nitro group is twisted by 36.7(2)° with respect to the mean plane of the indole ring. A weak intramolecular C–H···O hydrogen interaction is observed (C(9)···O(2), 2.935(5) Å; H(91)···O(2), 2.36; C(9)–H(91)···O(2), 117°). In the crystal packing the molecules are linked in dimers *via* N–H···O and C–H···O hydrogen bonds (N(1)···O(3)″, 2.801(5) Å; H(1N)···O(3)″, 1.93(3) Å; N(1)–H(1N)···O(3)″, 174(3)°. N(3)···O(1)″, 3.165(5) Å; H(3N)···O(1)″, 2.45(4) Å; N(3)–H(3N)···O(1)″, 153(3)°. C(12)···O(1)″, 3.280(5) Å; H(123)···O(1)″, 2.37 Å; C(12)–H(123)···O(1)″, 159°. Symmetry code:″ $= -1/2 + x, 1/2 - y, 1/2 + z$).

Conclusions

The results obtained in the present study clearly indicate that the reactivity of indoles with nitrogen dioxide is rather complex. The three kinds of indoles used (2- or 3-substituted and unsubstituted indole) show very different behaviour when reacted with nitrogen dioxide in an aprotic medium. Nitrosation is the main process occurring with 2-substituted indoles (unless an excess of nitrogen dioxide is used), oxidation with indole **2**, while nitration occurs with 3-alkyl substituted indoles. Oxidation and nitration may both be justified by a hydrogen atom transfer with formation of an indolyl radical followed by coupling with a nitrogen dioxide molecule, through the oxygen and the nitrogen atom, respectively. Hydrogen abstraction is a favoured process for indole **2** which may exist in equilibrium with the indoleninic tautomeric form (C3-H bond has a lower BDE value than N–H bond). Such a tautomeric equilibrium has more difficulty in occurring in the case of 2-substituted indoles **1a–b** and this may justify the fact that, for these compounds, electrophilic attack by N_2O_4 is the sole process observed. Indolyl radical (formed directly or through an electron transfer followed by deprotonation) is the key intermediate also in the reactions of indoles **3a–c** (at least when nitro derivatives are obtained). The occurrence of a particular reaction pathway, nitrosation instead of nitration or of oxidation, depends on the nature of the reacting indole.

When nitrous acid is used, electrophilic attack with formation of nitroso derivatives is the mechanism common to all the different indoles used, although nitroso indole **4** further reacts with nitrogen monoxide, derived from the decomposition of nitrous acid, affording the corresponding azo-bis-indole **7**.

The understanding of the complex reactivity of indoles is of crucial importance from a chemical and also a biological standpoint, since indoles are known to play an important role in biology and represent a frequently found motif amongst natural products.

Experimental

General

Melting points are uncorrected and were determined on an Electrothermal apparatus. IR spectra were recorded in the solid state on a Perkin Elmer MGX1 Spectrophotometer equipped with Spectra Tech. ^{1}H NMR were recorded at room temperature in $CDCl_3$ solution on a Varian Gemini 200 (TMS was taken as reference peak); *J* values are given in Hz. ^{1}H NMR spectra of compounds **23b,c** were also recorded on a Varian "Inova" operating at 600 MHz. UV spectra were recorded on a UV Kontron 941 spectrophotometer.

Gaseous nitrogen dioxide (purity 98.5%) was purchased from Fluka and used without purification. Nitrogen dioxide solutions were prepared by blowing the gas in a weighed flask while cooling with liquid nitrogen. The flask was weighed again after the gas was collected in the solid state and followed by addition of the solvent. The concentration of the solutions thus prepared was determined by considering the gas in the dimeric form (N_2O_4), as reported on the gas cylinder. Usually, they contained from 2×10^{-3} to 10^{-2} moles of N_2O_4 in 10 mL. Benzene was Carlo Erba RP-ACS grade. Indoles **1a**, **1b**, **2** and **3a** were purchased from Aldrich and used without further purification while indoles **3b**,**c** were synthesised according to the procedure described below starting from the commercially available tryptamine.

Reaction of indoles 1–3 with nitrogen dioxide. General procedure

The required indole (0.5 mmol) was dissolved in 5 mL of benzene and treated, at room temperature, with 0.5 mmol or 1 mmol of N_2O_4 dissolved in the same solvent. The reaction course was monitored by thin layer chromatography (TLC) using cyclohexane–ethyl acetate (8 : 2) as eluant. When an excess of N_2O_4 was used, the reactions were very fast and complete disappearance of the starting indole was observed as soon as the gas was added.

When a stoichiometric amount of N_2O_4 was used, the starting indole remained partially unreacted even when the reaction times were prolonged. For this reason, the reaction mixtures were worked up after 1 hour. The mixture was washed with $NaHCO_3$ (0.5 M) and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4 and evaporated to dryness. When the formation of a precipitate was observed, as with indoles **1a**,**b** and **2**, it was filtered, washed and dried, whereas the filtrate was washed with $NaHCO_3$ (0.5 M), extracted with ethyl acetate, dried over Na_2SO_4 and evaporated under reduced pressure.

The crude reaction mixtures were chromatographed by preparative TLC or by column chromatography and the isolated products were identified by comparing their spectroscopic data with those present in the literature for the same compounds or by comparison with authentic samples prepared according to the literature reports.

Reaction of indoles 1–3 with nitrous acid. General procedure

Sodium nitrite (2 mmol) was suspended into a benzene solution (10 mL) of the required indole (1 mmol) and the resulting mixture was thoroughly degassed with nitrogen. Acetic acid (10 mmol) suspended in 1 mL of benzene was added. The reaction course was monitored by TLC using cyclohexane–ethyl acetate (8 : 2) as eluant. Only in two cases, indoles **1a** and **2**, reactions were completed and the starting indole was completely consumed after 4 hours. After this time, the reaction mixture was neutralized with $NaHCO_3$ (0.5 M), extracted with ethyl acetate and dried over Na_2SO_4. If a precipitate was formed, it was filtered and washed with a methanol–water 1 : 1 mixture, whereas the filtrate was washed with $NaHCO_3$ (0.5 M), extracted with ethyl acetate, dried over Na_2SO_4 and evaporated under reduced pressure. If necessary, the crude reaction mixture was purified by column chromatography using cyclohexane–ethyl acetate (8 : 2) as eluant.

Compounds obtained were analyzed by NMR, IR and MS spectroscopy and, in some cases, by comparison with authentic samples.

1-Methyl-2-phenyl-3-nitroso-1*H*-indole[46] 4. Mp 212–213 °C (from ethanol); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 3.91 (3H, s, N–CH_3), 7.37–7.42 (m, 2H, arom.), 7.80–7.96 (m, 2H, arom.), 8.24–8.42 (m, 2H, arom.); m/z (EI^+) = 236 (M^+, 33%), 221 (45), 144 (100), 114 (55).

2-Phenyl-indole-3-one oxime[47] 5. The product was identified from its mass spectrum [m/z (EI^+) = 222 (M^+, 42%), 144 (100), 128 (34), 115 (65)] and compared with an authentic sample prepared according to the literature report.[47]

2-Phenyl-3-nitro-1*H*-indole[48] 6a. Mp 238–240 °C (from ethanol); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 7.39–7.43 (m, 3H, arom.), 7.52–7.55 (m, 3H, arom.), 7.70–7.75 (m, 2H, arom.), 8.28–8.34 (m, 1H, arom.), 8.55 (br s, 1H, N*H*); IR: ν_{max}/cm^{-1} 3254, 1525, 1359, 1213; λ_{max} (EtOH)/nm 227, 257 and 348 (lit.,[44] 229, 261 and 359).

1-Methyl-2-phenyl-3-nitro-1*H*-indole[49] 6b. Mp 118–119 °C (from petroleum ether); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 3.60 (s, 3H, N–CH_3), 7.44–7.49 (m, 5H, arom.), 7.55–7.58 (m, 3H, arom.), 8.37–8.43 (m, 1H, arom.); ν_{max}/cm^{-1} 1539, 1447, 1376, 1307, 1205; m/z (EI^+) = 252 (M^+, 58%), 234 (88), 222 (44), 105 (77), 84 (100).

3,3′-Azo-bis-1-methyl-2-phenylindole[50] 7. Mp 304–305 °C (from toluene); m/z (EI^+) = 440 (M^+, 31%), 410 (65), 256 (100), 178 (45) and by comparison with an authentic sample.[50]

2-Indolyl-3-one-3*H*-indole[51]8. Mp 218–219 °C (from benzene); ν_{max}/cm^{-1} 3149, 1725, 1625, 1598, 1325; m/z (EI^+) = 246 (M^+, 23%), 218 (35), 149 (52); λ_{max} (EtOH)/nm 273, 342 shoulder, 522.

2-Indolyl-3-oxime-3*H*-indole[51]9. Mp 244–245 °C (from petroleum ether); m/z (EI^+) = 261 (M^+, 19%), 246 (95), 218 (100); λ_{max} (EtOH)/nm 272, 334 shoulder, 350 shoulder, 445.

4-Nitro-*N*-acetyltryptamine 20b. Mp 132–133 °C (from diethyl ether); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 1.98 (s, 3H, C(O)–CH_3), 3.09 (t, 2H, J = 7.34, –CH_2–CH$_2$–NH), 3.49 (pq, 2H, J = 7.32, CH$_2$–CH_2–NH), 5.72 (br s, 1H, CH$_2$–N*H*), 7.18, (s, 1H, arom.), 7.26 (pt, 1H, J = 5.95, arom.), 7.66 (dd, 1H, J = 0.90 and 8.0, arom.), 7.87 (dd, 1H, J = 0.90 and 8.0, arom.), 9.01 (br s, 1H, N*H*-indole); ν_{max}/cm^{-1} 3154, 1644, 1504, 1261; m/z (EI^+) = 247 (M^+, 13%), 230 (20), 158 (99), 129 (100).

6-Nitro-*N*-acetyltryptamine 21b. Mp 150–151 °C (from diethyl ether); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 1.95 (s, 3H, C(O)–CH_3), 3.00 (t, 2H, J = 7.34, –CH_2–CH$_2$–NH), 3.60 (pq, 2H, J = 7.34, CH$_2$–CH_2–NH), 5.60 (br s, 1H, CH$_2$–N*H*), 7.34, (pd, 1H, J = 2.4, arom.), 7.62 (d, 1H, J = 8.92, arom.), 8.01 (dd, 1H, J = 2.04 and 8.82, arom.), 8.32 (pd, 1H, J = 1.86, arom.), 8.84 (bp, 1H, N*H*-indole); ν_{max}/cm^{-1} 3367, 1627, 1504, 1261; m/z (EI^+) = 247 (M^+, 2), 188 (100), 175 (42), 158 (22).

Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.31; H, 5.25; N, 17.05%.

1-Nitro-*N*-acetyltryptamine 22b. Oil; δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 2.01 (s, 3H, C(O)–CH_3), 2.86 (t, 2H, J = 7.2, –CH_2–CH$_2$–NH), 3.70 (pq, 2H, J = 7.2, CH$_2$–CH_2–NH), 6.98 (br s, 1H, CH$_2$–N*H*), 7.38–7.75 (m, 3H, arom.), 7.91 (m, 1H, H-4), 8.28 (d, 1H, J = 7.9, H-7); ν_{max}/cm^{-1} 3300, 1672, 1533, 1456, 1404, 1351, 1272; m/z (EI^+) = 247 (M^+, 2%), 201 (33), 157 (92), 130 (100).

1-Nitroso-3-methylindole[52] 23a. Oil; δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 2.28 and 2.32 (2s, 0.66 3H + 0.33 3H, CH_3), 7.31–7.59 (m, 3H, arom.), 7.91 (s, 0.25H, H-2), 8.15 (d, 0.5H, J = 6, H-4), 8.38 (m, 0.25H, H-7); ν_{max}/cm^{-1} 1453.5; m/z (EI^+) = 261 (21%), 233 (13), 160 (M+, 5), 130 (60).

1-Nitroso-*N*-acetyltryptamine 23b. Mp 120–121 °C (from benzene); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 1.98 and 1.99 (2s, 3H, C(O)–CH_3 C(O)–CH_3), 2.85–3.01 (m, 2H, –CH_2–CH$_2$–NH), 3.50–3.70 (m, 2H, CH$_2$–CH_2–NH), 5.75 (br s, 1H, CH$_2$–N*H*), 7.31–7.65 (m, 3H, arom.), 8.00 (s, 0.25H, H-2), 8.15 (d, 0.5H, J = 6 Hz, H-4), 8.30–8.40 (m, 0.25H, H-7); δ_H (600 MHz; $CDCl_3$; TMS; 25 °C) = 1.97 and 2.00 (2s, 0.66 3H + 0.33 3H, C(O)–CH_3 C(O)–CH_3), 2.94 and 2.99 (2t, 0.66 2H + 0.33 2H, J = 7.2, –CH_2–CH$_2$–NH), 3.60 (2q, 0.66 2H + 0.33 2H, J = 7.2, CH$_2$–CH_2–NH), 5.71 and 5.77 (2br s, 0.66 1H + 0.33 1H, CH$_2$–N*H*), 7.41–7.43 (m, 1.2 H, H-5 + H-6), 7.48–7.51 (pt, 0.8H, J = 7.2, H-5), 7.55–7.57 (m, 0.25H, H-7), 7.58 (s, 0.75H, H-2), 7.61 (d, 0.5H, J = 8.4, H-4), 8.03 (s, 0.25H, H-2), 8.17 (d, 0.5H, J = 7.8, H-4), 8.37–8.39 (m,

0.25H, H-7); ν_{max}/cm^{-1} 3285, 1640, 1564, 1444, 1143; m/z (EI^+) = 403 (80%), 344 (32), 231 (M^+, 9), 201 (100).

Anal. Calcd for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.45; H, 5.56; N, 18.25%.

1-Nitroso-*N*-benzoyltryptamine 23c. Mp 122–123 °C (from benzene); δ_H (200 MHz; $CDCl_3$; TMS; 25 °C) = 2.98–3.18 (m, 2H, –C*H*$_2$–CH$_2$–NH), 3.71–3.95 (m, 2H, CH$_2$–C*H*$_2$–NH), 6.29 (br s, 1H, CH$_2$–N*H*), 7.31–7.79 (m, 9H, arom.), 8.05 (s, 0.25H, H-2), 8.19 (d, J = 6 Hz, 0.5H, H-4), 8.32–8.41 (m, 0.25H, H-7); δ_H (600 MHz; $CDCl_3$; TMS; 25 °C) = 3.04 and 3.11 (2t, 0.66 2H + 0.33 2H, J = 7.5, –C*H*$_2$–CH$_2$–NH), 3.75 and 3.87 (2q, 0.66 2H + 0.33 2H, J = 7.5, CH$_2$–C*H*$_2$–NH), 6.58 and 6.60 (2br s, 0.66 1H + 0.33 1H, CH$_2$–N*H*), 7.34–7.44 (m, 4.2 H, arom.), 7.46–7.53 (m, 1/3 3H, arom.), 7.56–7.62 (m, 0.25H + 0.5H, H-7 + H-2), 7.65 (d, 0.5H, J = 9.8, H-2), 7.71–7.78 (2d, 2H, J = 8.2, arom), 8.03 (s, 0.25H, H-2), 8.17 (d, 0.5H, J = 9.8, H-4), 8.35–8.39 (m, 0.25H, H-7); ν_{max}/cm^{-1} 3343, 1638, 1444, 1145; m/z (EI^+) = 446 (10), 264 (38), 157 (14), 143 (100).

Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.66; H, 5.14; N, 14.45%.

Crystal structure of 2-(indol-3-yl)-3*H*-indol-3-one (8) and of 4-nitro-*N*-acetyl-tryptamine (20b)

Compound 8 (CCDC-608948). $C_{16}H_{10}N_2O$, M = 246.3, orthorhombic, a = 24.445(5), b = 4.319(2), c = 11.087(3) Å, V = 1170.5(7) Å^3, T = 298 K, space group $Pca2_1$ (no. 29), Z = 4, μ(Cu–Kα) = 0.716 mm^{-1}, brown plate, crystal dimensions 0.18 × 0.15 × 0.03 mm, 2545 reflections measured, 1165 unique (R_{int} = 0.032) which were used in all calculations. The final R and $wR(F_2)$ were 0.038 (for 787 observed data) and 0.088 (unique data). Although the compound does not contain atoms heavier than O, refinement of the Flack parameter led to a meaningless value of −0.3(7) (0.4(7) for the inverted structure) and, therefore, Friedel pairs were merged prior to the final refinement.†

Compound 20b (CCDC-608947). $C_{12}H_{13}N_3O_3$, M = 247.2, monoclinic, a = 7.399(2), b = 19.689(4), c = 8.576(2) Å, β = 108.374(4)°, V = 1185.7(5) Å^3, T = 298 K, space group $P2_1/n$ (no. 14), Z = 4, μ(Mo–Kα) = 0.102 mm^{-1}, yellow plate, crystal dimensions 0.17 × 0.12 × 0.04 mm, 9892 reflections measured, 1710 unique (R_{int} = 0.121) which were used in all calculations. The final R and $wR(F_2)$ were 0.043 (for 710 observed data) and 0.049 (all unique data). Due to the rather small plate crystals available, the intensity data were truncated to θ = 23.70°, at higher-angle data being weak and having poor internal agreement statistics. This could account for the rather low ratio unique–observed reflections (42%).†

Synthesis of *N*-acetyltryptamine 3b and *N*-benzoyltryptamine 3c

Tryptamine (10 mmol) was dissolved in 10 mL of pyridine and acetyl chloride or benzoyl chloride (12 mmol) was added dropwise at room temperature. The reaction mixture was stirred for 45 min then poured into 50 mL of water and stirred again for another 60 min. The reaction mixture was extracted with ethyl acetate, the organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. *N*-Acetyl or *N*-benzoyltryptamine was chromatographed on a silica gel column using ethyl acetate as the eluant.

N-Acetyltryptamine **3b**: δ_H (200 MHz, $CDCl_3$, TMS, 25 °C) = 1.91 (s, 3H), 2.97 (t, 2H, J = 6.2 Hz), 3.59 (q, 2H, J = 6.2 Hz), 5.61 (br s, 1H), 7.02 (s, 1H), 7.09–7.24 (m, 2H), 7.37 (d, 1H, J = 8 Hz), 7.61 (d, 1H, J = 8 Hz), 8.32 (br s, 1H); m/z (EI^+) = 202 (M^+, 15%), 143 (97), 130 (100).

N-Benzoyltryptamine **3c**: δ_H (200 MHz, $CDCl_3$, TMS, 25 °C) = 3.11 (t, 2H, J = 6.2 Hz), 3.81 (q, 2H, J = 6.2 Hz), 6.20 (br s, 1H), 7.08 (s, 1H), 7.12–7.24 (m, 2H), 7.36–7.45 (m, 4H), 7.65–7.69 (m, 3H), 8.18 (br s, 1H); m/z (EI^+) = 264 (M^+, 23%) 203 (24), 192 (66), 176 (100).

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