Sydnonimines as exogenous NO donors

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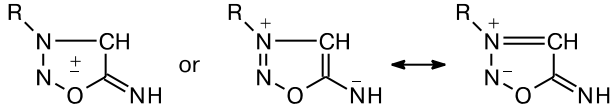
Chemical oxidation of a series of sydnonimine derivatives followed by NO release was studied. Substances having alkylamine substituents in the position 3 were shown to be considerably more potent NO donors in comparison with those having alkyl or aralkyl substituents in the position 3. It was suggested that the effect is mainly due to lowering of the activation energy of NO release upon stabilization of the cation formed competitively by the amino group.

Key words: sydnonimines, 3-aminosydnonimines, nitrogen oxide, NO donors.

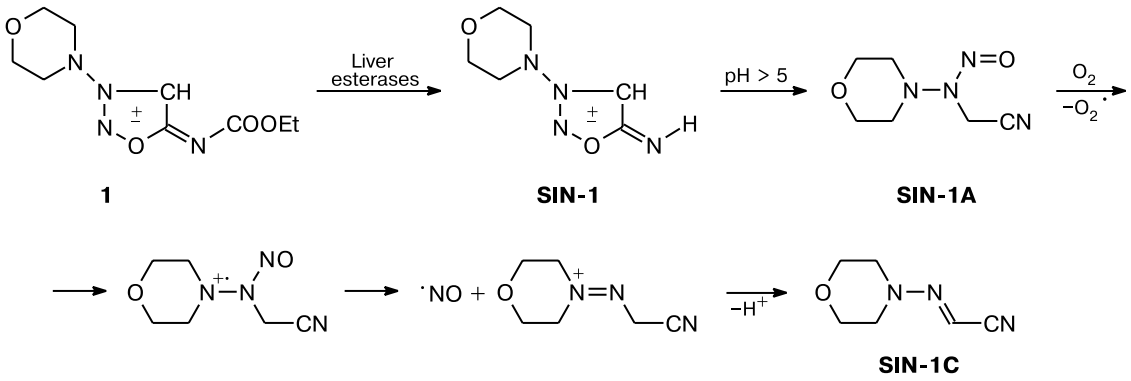
Sydnonimines represent a class of mesoionic heterocyclic compounds exhibiting a broad range of biological activities and capable of functioning as nitrogen oxide donors[1—3] (a review on the synthesis and properties of sydnonimines has been reported[4]).



For example, it has been found that the biological action of the known antianginal drug molsidomine (**1**) is related to its ability to release nitrogen oxide (Scheme 1).

Decomposition of **SIN-1A** (unlike that of **SIN-1**) is a pH-independent process and involves oxygen.[2] Oxygen consumption in buffered solutions of **SIN-1** is correlated with the formation of nitrogen oxide. It is significant that NO rather than HNO is formed from **SIN-1A** together with **SIN-1C**. The compound **SIN-1A** is the activator of soluble guanylate cyclase (sGC); it increases the intracellular concentration of cyclic guanosine monophosphate (cGMP) and, correspondingly, enhances its effects (vasodilation, inhibition of thrombocyte aggregation, neurotransmission, immune response regulation) typical of nitrogen oxide donors.[5] The drug can undergo either enzymatic or nonenzymatic degradation; under physiological or more alkaline conditions, **SIN-1** undergoes fast nonenzymatic ring cleavage to give **SIN-1A**, which is quite stable at pH 7.4 under anaerobic conditions in solutions protected from light. However, even traces of oxygen promote further transformations up to the formation of *N*-morpholinoiminoacetonitrile (**SIN-1C**).[6]

Scheme 1



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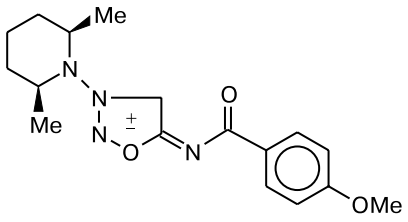
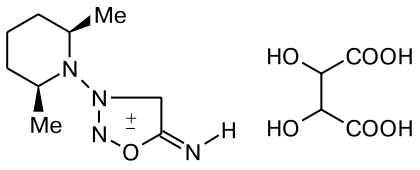
Worth noting are several other compounds of the sydnonimine series that attract considerable attention due to the high biological activity. These are molsidomine analogs such as pirsidomine, which is a potent vasodilator with prolonged action.[7—9] Like molsidomine, pirsidomine is first metabolyzed in the organism, and the metabolites function as nitrogen oxide donors.[7] The major pirsidomine metabolite is darsidomine (used as the tartrate), which causes selective dilatation of the coronary artery and has an anti-ischemic action without inducing tolerance on long-term administration.[10—12]



Pirsidomine



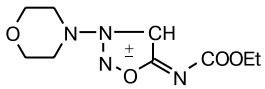
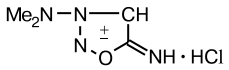
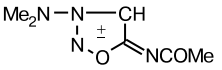
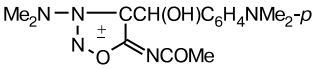
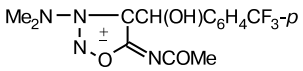
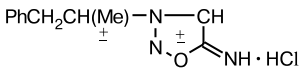
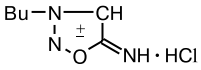
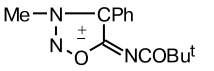
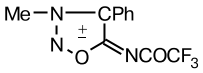
Darsidomine (tartrate)

Similarly to sydnonimines, derivatives of other mesoionic compounds also exhibit clear-cut nitrogen oxide donor properties and the corresponding biological effects. Solutions with pH 6.2—6.8 were shown[13] to be the optimal media for nitrogen oxide release from mesoionic compounds of the oxatriazolium structure. The mechanism of nitrogen oxide release proposed in the study cited[13] differs somewhat from that presented above. It was specially noted that the pathways of the *in vitro* and *in vivo* NO generation do not coincide; in the latter case, the release of nitrogen oxide can be enhanced due to the enzymatic degradation in the presence of thiols. Note that an alternative degradation route giving rise to the nitroxyl, HNO, instead of nitrogen oxide was also indicated for these compounds. It is also noteworthy that some known drugs, namely, the psychostimulant sydnocarb and the monoaminooxidase inhibitor sydnophen have also proved to be fairly active nitrogen oxide generators.[14,15]

Although a large number of publications have been devoted to the properties of sydnonimines,[15] the relationship between the NO-donor properties and the structures of these compounds has not been adequately elucidated.

The present work continues our research into the effect of the structures of sydnonimines on their chemical, hydrolytic, oxidative and, especially, NO-donor properties depending on the position and the nature of substituents in the sydnonimine ring. This may provide the base for the search for new biologically active compounds in the sydnonimine series.

Table 1. Yield of nitrogen oxide on chemical oxidation* of sydnonimine derivatives

Compound	Structure	Yield of NO (%)
1	 Molsidomine	14±2
2		31±2
3		19±2
4		22±2
5		22±2
6	 Sydnophen	1±0.2
7		0.7±0.2
8		0.7±0.2
9		3.6±0.2

* Oxidation conditions: 0.1 *M* NaOH, 80 °C.

The structures of compounds studied in this work are summarized in Table 1. As can be seen by examining their formulas, these compounds can be divided into two large groups: substances bearing amine-containing substituents (**1—5**) in position 3 of the sydnonimine ring, and those having alkyl substituents in this position (**6—9**). In both groups, some compounds contain acyl residues at the endocyclic nitrogen atom (**1**, **3—5** in the first group and **8**, **9** in the second group). In addition, the first group comprises compounds with arylalkyl substituents in position 4 (**4**, **5**), while compounds **8** and **9** belonging to the second group have a phenyl substituent at this position.

The properties of the sydnonimines as nitrogen oxide donors were compared in this study with the properties of molsidomine (**1**) and sydnophen (**6**). The substituted sydnonimines **2—5** and **7—9** were synthesized from the basic compounds of this series such as **2**, **3**, and 3-methyl-

4-phenylsydnonimine hydrochloride (**10**). Data on the preparation routes are given in the Experimental.

The relative intensity of NO release by each compound in a concentration of 10^{-4} mol L^{-1} was determined under oxidation by hydrogen peroxide or air oxygen in a phosphate buffer solution with pH 6.86 and in 0.1 *M* NaOH at various temperatures. Nitrogen oxide formed according to Scheme 1 is oxidized by water-dissolved oxygen (Scheme 2).

Scheme 2

$$2\,NO + O_2 \longrightarrow 2\,NO_2$$

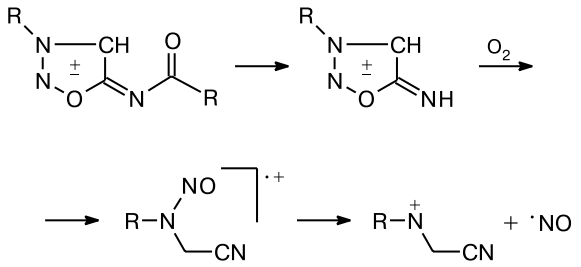
$$NO_2 + NO \xrightarrow{^-OH} 2\,NO_2^-$$

The NO_2^- anion was identified by the Griss reaction by a procedure[16] based on the use of sulfanilic acid, which is readily diazotized by the nitrous acid formed, and the resulting diazo compound undergoes the coupling reaction with *N*-(1-naphthyl)ethylenediamine to give an azo dye, which is determined by photometry (λ = 542 nm). Generally, this procedure provides rather reliable and reproducible results. By using a stronger oxidant, hydrogen peroxide, the yield of NO can be increased; however, in this case, NO_2^- is partially oxidized to NO_3^-. As a consequence, determination of NO by the procedure described above leads to underestimated and poorly reproducible results.

The results of oxidation of the compounds under study with air oxygen in a 0.1 *M* solution of NaOH at 80 °C for 30 min are summarized in Table 1.

It is quite evident that first-group compounds release much more nitrogen oxide upon the alkaline degradation. To interpret these results, let us turn back to the route of nitrogen oxide formation from sydnonimines, which includes hydrolysis of the *N-exo*-acyl group, ring cleavage, and NO liberation upon oxidation (Scheme 3).

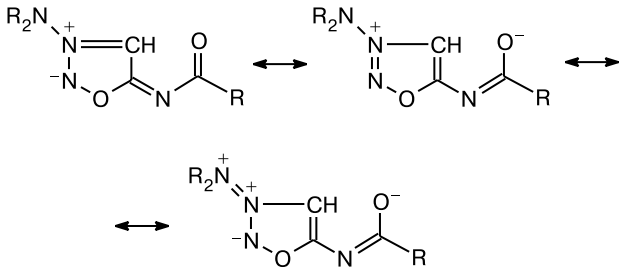
Scheme 3



I **II**

Comparison of the results for compounds **2** and **8** indicates that the degradation of the former produces an order of magnitude more nitrogen oxide. It is significant to emphasize that, unlike usual amine-substituted aromatic compounds where amino groups are electron donors, in sydnonimines, the amino group in position 3 is incapable of electron-donor conjugation. Indeed, structure **A** certainly does not make any significant contribution to the resonance hybrid (Scheme 4).

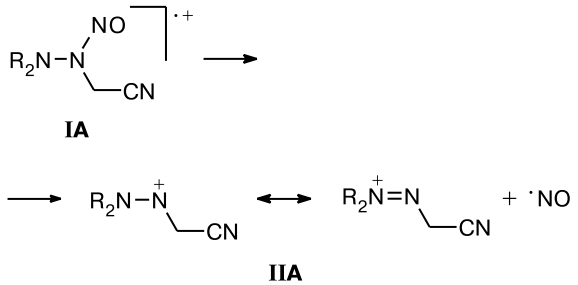
Scheme 4



A

Therefore, it appears probable that the first hydrolytic step, elimination of the *N*-acyl group under the action of the hydroxide anion, proceeds more easily in the presence of a dialkylamino group than the electron-donating alkyl substituent. However, it must be admitted that this effect does not predominate. Otherwise, it would be impossible to explain why the release of NO from compounds containing no *N-exo*-acyl groups (for example, **2** and **6** or **7**) is also dramatically different. In our opinion, the crucial role in this difference is played by the transformation of radical cation **IA** into cation **IIA** and nitrogen oxide. In cation **II**, the situation differs crucially from that in the initial molecule and the dialkylamino groups are now potent electron donors (Scheme 5).

Scheme 5



IA

IIA

If we assume that the transition state of the **IA** → **IIA** process is similar to the final compound (**IIA**), this stabilization should result in a sharp decrease in the free activation energy of the process and, hence, in acceleration of the degradation and a decrease in the contribution of side (as regards NO formation) reactions such as nitroxyl

formation considered above and the known[17] ring transformation of sydnonimines into oxotriazoles.

It is noteworthy that the results obtained in this study are in good agreement with the tentative results obtained in the study of activation of the enzyme sGC by these compounds (the biochemical studies were carried out by prof. I. S. Severina at the Research Institute of the Biomedical Chemistry of the Russian Academy of Medical Sciences, the results will be published elsewhere). Soluble guanylate cyclase (sGC) catalyzes the biosynthesis of so-called secondary messenger, *i.e.*, cyclic guanosine monophosphate from guanosine triphosphate.

Study of various preparations on sGC is a convenient and illustrative test to verify the ability of substances to release nitrogen oxide, as the non-heme mechanism of sGC activation is exceptionally seldom found. In our case, sGC activation by first-group compounds exceeds appreciably the activation caused by the second-group sydnonimines.

The results obtained imply that the nitrogen oxide donors most efficient in this series should be selected among compounds containing amino substituents in position 3 of the sydnonimine ring, which may prove to be a rational approach to the search for new biologically active compounds.

Experimental

^{1}H NMR spectra (DMSO-d_6) were recorded on a Bruker WM-400 spectrometer. Melting points were determined in a glass capillary in a metallic block and were not corrected. All reactions involving organometallic compounds were carried out under dry argon in anhydrous solvents. The absorbance of solutions with an azo dye were measured on a KFK-3-01 photometer at a wavelength of 542 nm.

3-Methyl-4-phenylsydnonimine hydrochloride (**10**),[18] 3-butylsydnonimine hydrochloride (**7**),[19] 3-dimethylaminosydnonimine hydrochloride (**2**),[20] and 3-dimethylamino-*N*(6)-acetylsydnonimine (**3**)[20] were synthesized by previously described procedures.

3-Methyl-4-phenyl-*N*(6)-pivaloylsydnonimine (8). Pivaloyl chloride (2.46 mL, 2.4 g, 0.02 mol) was added to a suspension of compound **10** (2.12 g, 0.01 mol) in 10 mL of THF and the mixture was cooled to 0 °C. Pyridine (4.04 mL, 3.95 g, 0.05 mol) was added dropwise. The reaction mixture was stirred for 5 h at 0 °C and allowed to stand for ~14 h. The solvent was evaporated *in vacuo* and the residue was treated with 20 mL of water and 30 mL of chloroform. The layers were separated, the aqueous layer was extracted with chloroform (2×15 mL), and the combined organic extracts were dried with sodium sulfate and concentrated. The residue was recrystallized from ethanol to give 2.33 g (90%) of compound **8**, m.p. 149—150 °C. Found (%): C, 64.55; H, 6.81; N, 15.90. $C_{14}H_{17}N_3O_2$. Calculated (%): C, 64.85; H, 6.61; N, 16.20. ^{1}H NMR, δ: 1.26 (s, 9 H, Bu^t); 4.23 (s, 3 H, Me); 7.50—7.60 (m, 3 H, *p*- and *m*-H, Ph); 7.66—7.72 (m, 2 H, *o*-H, Ph).

3-Methyl-4-phenyl-*N*(6)-trifluoroacetylsydnonimines (9). Trifluoroacetic anhydride (2.8 mL, 4.2 g, 0.02 mol) was added to a suspension of 3-methyl-4-phenylsydnonimine (2.12 g, 0.01 mol) in 10 mL of THF and cooled to 0 °C. Pyridine (2.73 mL, 2.0 g, 0.02 mol) was added dropwise to the resulting mixture. The mixture was stirred for 5 h at 0 °C and allowed to stand for ~14 h. The solvent was evaporated *in vacuo*, the residue was treated with 20 mL of water and 30 mL of chloroform. The layers were separated, the aqueous layer was extracted with chloroform (2×15 mL), and the combined organic extracts were dried with sodium sulfate and concentrated. The residue was recrystallized from ethanol to give 2.30 g (85%) of compound **9**, m.p. 183—184 °C. Found (%): C, 48.93; H, 2.55; N, 15.00. $C_{11}H_8F_3N_3O_2$. Calculated (%): C, 48.72; H, 2.97; N, 15.49. ^{1}H NMR, δ: 4.35 (s, 3 H, Me); 7.57—7.69 (m, 5 H, Ph).

4-Hydroxy-3-dimethylamino-(4-dimethylaminophenyl)methyl-*N*(6)-acetylsydnonimines (4). A 1.6 *M* solution of Bu^{n}Li in hexane (1.84 mL, 2.95 mmol) was added to a solution of compound **3** (0.5 g, 2.95 mmol) in 20 mL of THF at −78 °C. The mixture was stirred for 30 min and 4-dimethylaminobenzaldehyde (0.57 g, 3.82 mmol) was added to the resulting red-brown solution. The mixture was stirred for 15 min at −78 °C and cooling was terminated. After the reaction mixture warmed up to room temperature, water (1 mL) was added. The solvent was removed *in vacuo* and the residue was dissolved in chloroform and filtered through an Al_2O_3 layer (2×3 cm), the product being eluted with chloroform. Chloroform was evaporated *in vacuo* and the residue was chromatographed on a SiO_2 plate (elution with a chloroform—ether mixture (5 : 1)). Recrystallization from an ether—hexane mixture (1 : 2) gave 0.66 g (70%) of compound **4**, m.p. 123—124 °C. Found (%): C, 56.22; H, 6.40; N, 21.50. $C_{15}H_{21}N_5O_3$. Calculated (%): C, 56.41; H, 6.63; N, 21.93. ^{1}H NMR, δ: 1.86 (s, 3 H, Me); 2.92 (s, 6 H, C_6H_4—N(C$\underline{H}_3$)$_2$); 3.16 (s, 6 H, 4-NMe_2); 5.93 (s, 1 H, C$\underline{H}$—OH); 6.50—6.60 (m, 1 H, CH—O$\underline{H}$); 6.67, 7.10 (both m, 2 H each, C_6H_4).

4-Hydroxy-3-dimethylamino-(4-trifluoromethylphenyl)methyl-*N*(6)-acetylsydnonimine (5). A 1.6 *M* solution of Bu^{n}Li in hexane (1.84 mL, 2.95 mmol) was added to a solution of compound **3** (0.5 g, 2.95 mmol) in 20 mL of THF at −78 °C. The mixture was stirred for 30 min and 4-trifluoromethylbenzaldehyde (0.57 g, 3.82 mmol) was added to the resulting red-brown solution. The mixture was stirred for 15 min at −78 °C and cooling was terminated. After the reaction mixture warmed up to room temperature, water (1 mL) was added. The solvent was removed *in vacuo* and the residue was dissolved in chloroform and the solution was filtered through an Al_2O_3 layer (2×3 cm), the product being eluted with chloroform. Chloroform was evaporated *in vacuo* and the residue was chromatographed on a SiO_2 plate (elution with a chloroform—ether mixture (5 : 1)). Recrystallization from an ether—hexane mixture (1 : 2) gave 0.76 g (75%) of compound **5**, m.p. 96—97 °C. Found (%): C, 48.91; H, 4.52; N, 16.33. $C_{14}H_{15}N_4F_3O_3$. Calculated (%): C, 48.84; H, 4.39; N, 16.27. ^{1}H NMR, δ: 1.80 (s, 3 H, Me); 3.31 (s, 6 H, NMe_2); 6.13 (s, 1 H, C$\underline{H}$—OH); 7.43, 7.70 (both m, 2 H each, C_6H_4).

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