Oximes, amidoximes and hydroxamic acids as nitric oxide donors

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Quaternized pyridine aldoximes (2- and 4-PAM), some hydroxamic acids and amidoximes produce NO under mild oxidation and activate soluble guanylate cyclase.

Recently we have reported that some oximes of 2-arylmethylene- and 2-arylmethylquinuclidin-3-one gave NO and the parent ketones under mild oxidation. The most active NO donors containing a 2-HO-phenyl group proximal to the oxime fragment activated soluble guanylate cyclase; 4-HO-isomers, 2-MeO- and 4-MeO-derivatives as well as the oxime of quinuclidin-3-one were much less active.[1–2]

The aim of this work is an investigation of the factors affecting the ability of the oxime group to serve as NO precursor, with special attention given to the influence of its conjugation with heteroatoms (O, N) and aromatic rings. For this purpose three series of compounds: oximes RCH=NOH **1** and **2**, hydroxamic acids RC(=O)NOH **3** and amidoximes $RC(NH_2)$=NOH **4** were chosen. Methyl derivatives **1a**, **3a**, **4a** were used as the reference compounds and aromatic substituents in the above series were designed for an elucidation of the possible role of H-bonding (R: **b**, Ph; **c**, 2-HO; **d**, 2-MeO; **e**, 4-HO; **f**, 4-MeO-phenyl; **h**, 2-Py, **i**, 4-Py; **j**, 2,6-Py) through interaction at the enzyme site at physiological pH values. Quaternized pyridine aldoximes **2h**,**i** served as strongly electron-deficient nuclei lacking H-bond acceptor properties.

The required compounds were obtained according to the published procedures from hydroxylamine and the corresponding aldehydes for *syn*-aldoximes[3] [**1a**: bp 113–114 °C (lit.,[4] 112–114 °C); **1b**: mp 29–31 °C (lit.,[5] 35 °C); **1c**: mp 58–59 °C (lit.,[6] 56–57 °C); **1d**: mp 90–92 °C (lit.,[5] 92 °C); **1e**: mp 62–65 °C (lit.,[7] 68–70 °C); **1f**: mp 52–54 °C (lit.,[3] 61–62 °C)], or the corresponding esters for hydroxamic acids [**3d**: mp 132–134 °C (acetone–diethyl ether, lit.,[8] 129–131 °C); **3e**: mp 179 °C (H_2O, lit.,[9] 168 °C); **3f**: mp 159–160 °C (acetone–hexane, lit.,[9] 162 °C); **3h**·0.5H_2O: mp 136 °C (H_2O, lit.,[10] 120 °C, anhydrous); **3i**: mp 175 °C (H_2O, lit.,[10] 161 °C); pyridine-2,6-dihydroxamic acid **3j**·0.5H_2O: mp 185 °C (H_2O, lit.,[10] 217 °C, anhydrous)] or the corresponding nitriles for amidoximes [**4h**: mp 113–114 °C (H_2O, lit.,[11] 115 °C); **4i**: mp 205 °C (H_2O, lit.,[11] 199 °C); pyridine-2,6-diamidoxime **4j**: mp 235–237 °C (H_2O, lit.,[12] 214 °C)]. Commercial preparations of **1h** (Merck), **1i** (Lancaster), **2a** (Aldrich) and **3b**,**c** (Reakhim, Russia) were used. Quaternization of **1h**,**i** with MeI was carried out in MeOH at room temperature. Amidoximes **4a**,**b**,**g** and acetamidine hydrochloride were a gift from Dr S. M. Vinogradova. According to ^{1}H NMR spectroscopy the purity of all samples was more than 98% and compounds **1e**,**f** contained *ca.* 5% of *anti*-isomers.

The results of oxidation of the above compounds with $K_3[Fe(CN)_6]$ are given in Table 1. NO was trapped and determined as $[Fe(CN)_5NO]^{2-}$ (nitroprusside) anion by pulsed differential polarography according to the previously described protocol.[1–2] Under these conditions oximes **1** do not generate NO, which is in sharp contrast with our earlier finding that (*E*)-2-[(2′-hydroxyphenyl)methylene]- and 2-[(2′-hydroxyphenyl)methyl]quinuclidin-3-one gave NO in 17% and 10% yields, respectively. A possible explanation for this difference may lie in the fact that the rigid structure of the quinuclidine derivatives allows for intramolecular interaction of the 2′-OH group and the oxime fragment, which is impossible for *syn*-oxime **1c**. Even so, the absence of any difference within the series **1**, comprising both the strong acceptor pyridine and strong donor hydroxyphenyl rings, as well as the non-conjugated methyl group, is rather surprising. Probably, the competition between the donor–acceptor properties of R and the acidities of oxime and phenolic OH groups, responsible for the proportion of oxime anions, accounts for the phenomenon.

Table 1 Yield of NO (%) from oximes, amidoximes and hydroxamic acids: oxidation (c = 2×10^{-4} M, 4% EtOH–H_2O) by $K_3[Fe(CN)_6]$ at pH 12.[a]

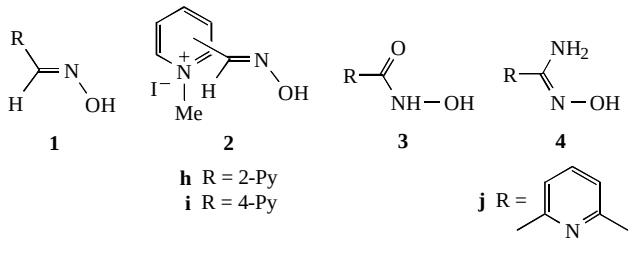
R	Compound	**1**	**2**	**3**	**4**
Me	**a**	0.0	—	22.9	25.0
Ph	**b**	0.5	—	9.0	10.0
2-HOC_6H_4	**c**	0.0	—	14.0	—
2-$MeOC_6H_4$	**d**	0.0	—	5.1	—
4-HOC_6H_4	**e**	0.0	—	6.5	—
4-$MeOC_6H_4$	**f**	0.0	—	16.4	—
4-$HOC_6H_4CH_2$	**g**	—	—	—	5.5
2-Py	**h**	0.0	6.4	0.0	20.6
4-Py	**i**	7.4	62.2	0.8	29.5
2,6-Py[b]	**j**	—	—	3.1	40.0

[a] $Na_2[Fe(CN)_5NO]$ 100%, NH_2OH·HCl 55.8%. [b] 2,6-Disubstituted pyridyl.

The difference is more pronounced for hydroxamic acids **3**, which are completely ionized at the pH employed[13–15] (Table 2). Among them the most active NO donor proved to be acetohydroxamic acid **3a**. The pyridine ring essentially inhibited the activity **3h**–**j**, though **3j** shows some residual activity. The absence of any visible trend in the aromatic sub-series **3b**–**f** allows us to suggest the operation of several opposing factors. In any case, the involvement of a formal oxime group in conjugation with the oxygen lone pair (hydroxamic acids exists as **3** and not **11**) in **3a**–**f** leads to a marked increase in activity compared to the corresponding oximes **1a**–**f**, **3a** being half as active as NH_2OH·HCl.

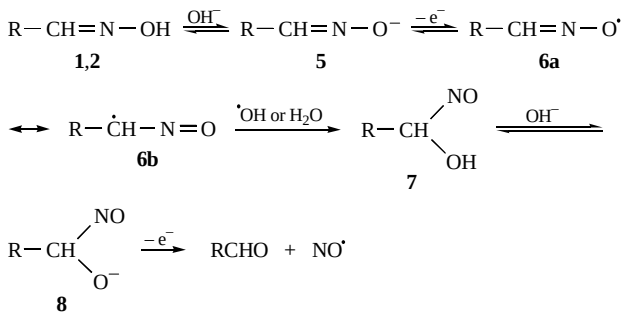
The simple amidoximes **4a**,**b** essentially mimic the behaviour of the corresponding hydroxamic acids **3a**,**b**. At the same time, the activity of non-conjugated phenylacetic derivative **4g**, is only half of that of benzamidoxime **4b** instead of the expected value between **4a** and **4b**. In contrast to hydroxamic acids, the substitution of a benzene ring for pyridine leads to a sudden leap in amidoxime activity. The values for compounds **4h**,**i** are close to the value for **4a**. The activity of more conjugated **4i** is 1.5 times higher than that of **4h** and pyridine-2,6-diamidoxime **4j** is twice as active as its 2-substituted prototype **4h**.

The pathways leading to NO generation from oximes, amidoximes and hydroxamic acids are to be investigated separately but the observed facts may be rationalized as



1 **2** **3** **4**

Scheme 1



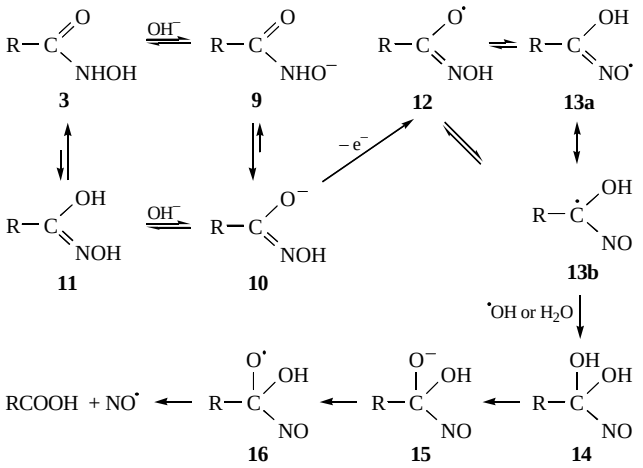
Scheme 2

follows. At pH 12 all the oximes (except **1a**) and hydroxamic acids studied are ionized > 90% (Table 2) and the first step in the oxidation is undoubtedly the loss of an electron from their anion (Scheme 2). Within the series of oximes **1a**–**i**, **2h**–**j** only acceptor pyridine rings provide the favourable combination of an increase in content of **5** and in radical **6** (presented in two resonance structures **6a** and **6b**) the stabilisation responsible for the subsequent NO generation. The absence of a correlation between **1h**,**i**, **2h**,**i** acidity and the yield of NO shows the importance of the stabilisation. The latter is higher for 4-Py than for the 2-Py substituent, 1-methyl-4-pyridinealdoxime **2i** being the most active of all the compounds tested. Nor can the adverse contribution of *ortho*-effects in **1h**, **2h**, especially steric shielding by N–Me in **2h**, be excluded. A control experiment demonstrated that **4h**,**i** are stable at pH 12, *i.e.* NO formation is not caused by hydroxylamine release due to hydrolysis or other processes.

The behaviour of hydroxamic acids is essentially the same (Scheme 3). Both predominant oxo-form **3** and minor imino-form **11** give the better conjugated (compared to **9**) imino-anion **10**, converting into tautomeric radicals **12**,**13**, of which the latter is involved in reactions similar to that of **6**. TLC examination of the reaction mixtures originating from **3b**,**c**,**f** reveals only the presence of the corresponding carboxylic acids. No detectable amounts of the products of ring hydroxylation or radical dimerisation have been found.

Amidoximes are not so straightforward. Though their acidity is low and their basicity is close to pyridine (pK_a: **4a** 5.95, **4b** 5.10, Py 5.10[18]), they form salts in water not only with acids but also with alkalis.[19] So, in this case both the anion of **4** can be oxidized into **18** and the neutral amidoximes can give cation radical **17** followed by deprotonation to **18** (Scheme 4). The reactive **18** can behave similarly to the above mentioned key-radicals **6** and **13** (Schemes 2 and 3), forming NO *via* N,O-intermediate **19**, but this nevertheless seems not to be the principal route, if at all.

Despite the fact that **4b** gives benzamide and some benzoic acid as the end-products (TLC), the first detectable reaction product is PhCN (GC-MS). According to the literature the



Scheme 3

Table 2 Ionization of oximes and hydroxamic acids in water at pH 12 and room temperature.

Compound	**1**		**2**		**3**	
	pK_a	% of anion	pK_a	% of anion	pK_a	% of anion
a	12.4[a]	28.47	—	—	9.46[13]	99.71
b	10.68[5]	95.43	—	—	8.75[15]	99.94
c	9.3[16]	99.80	—	—	7.43[13]	99.997
d	10.88[5]	92.95	—	—	—	—
f	10.92[5]	92.32	—	—	9.0[14]	99.90
h	10.14[17]	98.64	8.0[17]	99.99	8.7[14]	99.95
i	9.99[17]	99.03	8.57[17]	99.96	7.8[14]	99.994

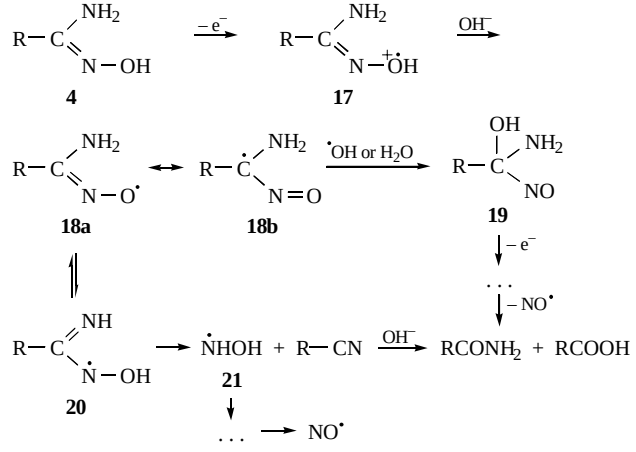
[a]For Me_2C=NOH. % of anion = $100/[1 + 10^{(pK_a - pH)}]$.

latter is formed from **4b** and strong oxidizers, while under conditions similar to ours a low yield of 5-amino-4,5-dihydro-3,5-diphenyl-1,2,4-oxadiazole was obtained.[20] On mixing of 0.11 mmol of **4b**, 0.3 mmol of $K_3[Fe(CN)_6]$ and 0.9 mmol of KOH in 0.5 ml of water at 80 °C the initially clear solution almost immediately becomes opaque (emulsion of hydrophobic PhCN) and a strong smell of bitter almond can be observed. After 2–3 min the solution becomes clear again and contains no **4b**. A parallel experiment without $K_3[Fe(CN)_6]$ showed that after 5 min only about half of **4b** was hydrolyzed to benzamide. We have no exact kinetic data but the consumption of **4b** in the presence of $K_3[Fe(CN)_6]$ is much faster than the alkaline hydrolysis leading to benzamide and hydroxylamine, *i.e.* elimination of PhCN from some radical intermediate (probably **18** → **20** → **21**) but not simple hydrolysis is the main source of NO from amidoximes. The radical **21** or its anion have been reported as NO precursors under these conditions.[21]

General considerations do not allow us to exclude oxidation of the amidoxime NH_2 group instead of NOH as the first step, but the much more basic and more electron-rich $MeC(=NH)NH_2$ does not give NO under the conditions used, which supports the suggested scheme.

The two-fold drop in activity upon substitution of Me for Ph in **3a**/**3b** and **4a**/**4b** can be attributed to a decrease in reactivity of the radicals stabilized by the aromatic ring. Further manifestation of this effect aggravated by a reduction in the electron density on anion **10**, might be responsible for the low activity of pyridyl hydroxamic acids **3h–j**. Since the nature of R has very little influence on the acidity of the =NOH group in amidoximes,[18] the acceptor properties of pyridyl substituents are not as important in steps **4** → **17** → **18**, but they certainly play a role in the subsequent stages, which may account for the lack of a completely parallel route in the series **3** and **4**.

Preliminary tests[22] show that about a two-fold activation of soluble guanylate cyclase from human platelets is achieved by **3a**,**c** and **4h** at a concentration 10^{-6} mol dm^{-3}. **3e** (4-OH isomer of **3c**) is less active (×2.5 at 10^{-5} mol dm^{-3}) and OMe-derivatives of OH-acids **3d**,**f** are not active at all. **3b**, **4a**,**b** give a *ca.* 1.5-fold activation at 10^{-4}–10^{-5} mol dm^{-3}, while



Scheme 4

Table 3 Activation of soluble guanylate cyclase (2 series) relative to control = 1.

Compound	Concentrations tested/mol dm^{-3}			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
	Series 1			
1h	1.3	2.0	1.6	—
2h	1.4	2.1	1.1	—
2i	1.1	1.6	1.5	—
$NH_2OH·HCl$	1.1	1.3	2.1	—
	Series 2			
4a	—	1.4	1.3	1.3
3b	—	1.4	1.7	1.5
4b	—	1.4	1.3	1.2
$NH_2OH·HCl$	—	2.5	2.2	3.6

pyridine aldoximes **1h**, **2i**,**h** give a 'bell-shaped' curve with a maximum at *ca.* 10^{-5} mol dm^{-3} (Table 3). In general, enzyme activation data for the hydroxamic acids do not contradict their behaviour under chemical oxidation (Table 1), but the influence of an aromatic substitution pattern in **3b–f** is strikingly similar to that observed for oximes of 2-arylmethylene-quinuclidin-3-ones[1,2] (see above). Consequently, at a physiological pH compatible with the existence of intramolecular hydrogen bonds **22**, the 2-OH-phenyl group provides either superior stabilisation of the intermediates (or the released NO) or higher complementarity to the active site, *i.e.* we can see the pronounced *ortho*-effect in **3c**. The data for pyridine aldoximes also suggest the involvement of some *ortho*-interaction or site complementarity, since 2-pyridyl-substituted oximes **1h**, **2h** activate soluble guanylate cyclase better than 4-substituted **2i** and, at the lower concentrations, better than hydroxylamine (Table 3).

Since the preparation of soluble guanylate cyclase used is not free from non-specific oxidative enzymes also capable of producing NO, we cannot tell whether these *ortho*-effects are connected with the complementarity to soluble guanylate cyclase itself, to non-specific oxidative enzymes (P-450 *etc.*) or to an increase in the lifetime of NO released by non-specific enzymes, which allows NO to reach the soluble guanylate cyclase more efficiently and to cause its activation. Such stabilisation of NO by *N*-hydroxyguanidine (**23**, R = H), its derivatives and L-*N*-hydroxyarginine (L-NOHA) **24** is well documented.[23,24] It should be noted that anions of oximes do not interact with NO.[25]

Thus, although oxidation of =N–OH-containing substrates (or their tautomers **3**) at pH 12 cannot be considered as truly biomimetic, this very simple method allows us to carry out a fast preliminary screening of potential NO precursors. As a result, we were able to reveal the unknown NO generating potential of the well-known oximes, amidoximes and hydroxamic acids (there is only one brief mention of NO formation from *N*-hydroxybenzenesulfonamide: Piloty acid, the rate of which correlates with the rate of hydroxysulfonamide hydrolysis[26]) and some correlation between the chemical oxidation and soluble guanylate cyclase activation for these hydroxylamine derivatives. Though the better NO yields and values of soluble guanylate cyclase activation achieved *in vitro* are comparable to that of hydroxylamine itself, the use of the three classes of readily available new NO donors as templates for design of NO releasing 'pro-drugs' with desired *in vivo* properties looks rather promising. One more argument in favour of such a suggestion lies in the similarity of the observed chemistry and NO biosynthesis by nitric oxide synthase from L-arginine **23** *via* L-NOHA **24** followed by formation of NO and L-citrulline **25**[27,28] (oxidation of simpler *N*-hydroxyguanidines is quite complicated[29]).

In conclusion, we cannot avoid mentioning that hydroxylamine, hydroxamic acids and especially quaternary pyridine aldoximes **2h** (2-PAM) and **2i** (4-PAM) were the first efficient antidotes against nerve gas poisoning.[8,10,14,15,17] The commonly accepted 'nucleophilic' mechanism of acetylcholine esterase reactivation could not explain all the phenomena observed *in vivo*, which may account for the potential NO contribution. The last hypothesis is far beyond the scope of this work and must be investigated separately.

This work was supported by the Russian Foundation for Basic Research (grant no. 96-04-48325).

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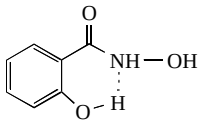

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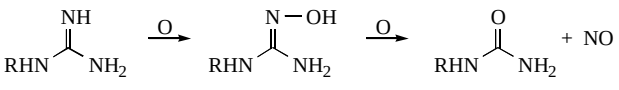
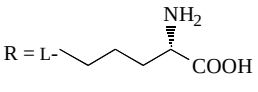


23 **24** **25**



Scheme 5

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Received: Moscow, 28th October 1997
Cambridge, 15th December 1997; Com. 7/07978H