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FORMATION OF NITROGEN OXIDES INCLUDING NO FROM OXIDATIVE CLEAVAGE OF C=N(OH) BONDS : A GENERAL CYTOCHROME P450-DEPENDENT REACTION.

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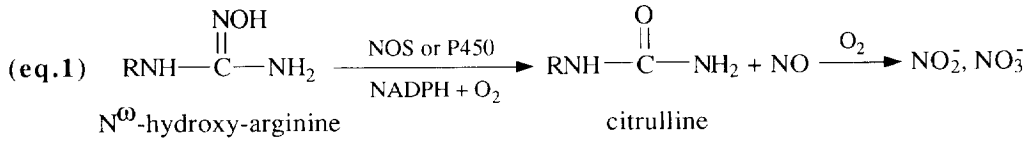
Abstract : Several compounds such as aldoximes, ketoximes, amidoximes and guanidoximes were oxidized by liver microsomes from dexamethasone-treated rats with formation of nitrogen oxides such as NO_2^-, NO_3^- and NO. The oxidative cleavage of their C=N(OH) bond appears as a general P450 3A-catalyzed reaction, which is particularly efficient in the case of N,N'-disubstituted guanidoximes.

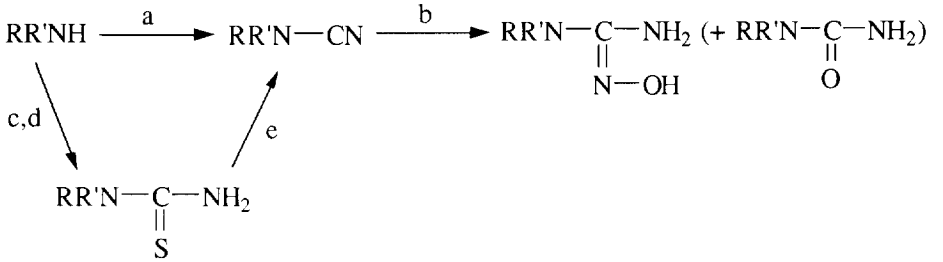
Nitric oxide, NO, is a recently discovered biological effector which plays a key role in the cardiovascular and central and peripheric nervous systems, and modulates immunological responses[1]. *In vivo*, NO is biosynthesized by a two-step oxidation of L-arginine, the second step being an oxidative cleavage of the C=N(OH) bond of intermediate N^{ω}-hydroxy-L-arginine (eq.1)[2,3]. This oxidation is catalyzed by NO synthases that are hemeproteins closely related to cytochromes P450[3]. Even classical rat liver P450s catalyse the oxidation of N^{ω}-hydroxy-L-arginine to citrulline and nitrogen oxides including NO[4].



Recently, we found that an exogenous compound, benzamidoxime **IIIc**, could also generate nitrogen oxides including NO by a P450-dependent oxidation of its C=N(OH) bond[5]. Moreover, the P450-dependent oxidation of a guanidoxime, N-hydroxydebrisoquine, to the corresponding urea was recently reported[6]. These results suggested that exogenous compounds containing a C=N(OH) function could act as precursors of NO and be exogenous equivalents to N^{ω}-hydroxy-L-arginine for NO formation. In order to test the generality of this phenomenon, we have synthesized various compounds containing a C=N(OH) function such as alkyl- and aryl-aldoximes **I**, ketoximes **II**, amidoximes **III** and guanidoximes **IV**, and compared their capacity to generate NO_2^- and/or NO_3^- (the final, stable oxidized derivatives of NO) upon oxidative cleavage of their C=N(OH) bond by rat liver cytochromes P450. This communication reports that the 18 compounds studied are oxidized by liver microsomes with formation of nitrogen oxides, and shows for the first time that the P450-catalyzed oxidative cleavage of C=N(OH) bonds is a general reaction.

Synthesis[7]

Aldoximes **I**, ketoximes **II** and amidoximes **III** (Table 1) were prepared from reaction of NH_2OH, HCl with the corresponding aldehydes, ketones and nitriles by classical methods[8,9]. The synthetic routes used to prepare mono- or N,N-disubstituted guanidoximes from the corresponding cyanamides are outlined in Scheme 1. Addition of NH_2OH, HCl to intermediate cyanamides gave guanidoximes **IV** as well as small amounts of ureas which could be easily separated. Symetrically N,N'-disubstituted guanidoximes were obtained upon reaction of NH_2OH, HCl with the corresponding carbodiimides[10].

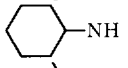
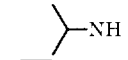
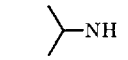
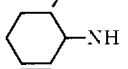
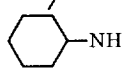
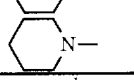


Scheme 1 : Synthetic routes to guanidoximes IV (Table 1).
a) 1.1 eq. BrCN, 2eq. NEt_3, Et_2O, 3h, O°C[11] ; b) 1.5 eq. NH_2OH, HCl, EtOH, 15h, reflux ; c) 1 eq. PhCOCl, 1.05 eq. NH_4SCN, acetone, 0.5h, 0°C then 1 eq. RNHR', 1h, reflux ; d) NaOH, 1h, reflux then HCl[13] ; e) 1 eq. $Pb(OAc)_2$, H_2O, 0.5h, 70°C[12].

Oxidation by rat liver cytochromes P450.

The ability of the various compounds containing a C=N(OH) function to undergo a P450-dependent oxidative cleavage of their C=N bond was first studied by incubating them with rat liver microsomes in the presence of the cofactors of cytochromes P450, NADPH and O_2, and by measuring the formation of NO_2^- [14]. Table 1 shows that , for all the compounds studied, liver microsomes from rats treated by dexamethasone were much more active than microsomes from untreated rats. This result is in complete agreement with previous results reported for amidoxime **IIIc**, showing that cytochromes P450 from the 3A subfamily which are specifically induced by dexamethasone are the most active ones[5]. All aldoximes **I**, ketoximes **II**, amidoximes **III** and guanidoximes **IV** studied led to significant amounts of NO_2^-. Its formation was clearly dependent on a cytochrome P450-catalyzed oxidation as it did not occur either with boiled microsomes or in the absence of NADPH, and was greatly decreased in the presence of troleandomycin, a classical inhibitor of P450 3As (data not shown, see also ref. 5, 4, 15). Moderate formation of NO_2^- (between 2 to 7 nmol per nmol P450 in 10 min) was observed for aldoximes, ketoximes and amidoximes, whereas much higher activities were obtained for guanidoximes **IV** (between 11 to 79 nmol.nmol $P450^{-1}$. $(10\ min)^{-1}$). In that regard, it is noteworthy that the rates of NO_2^- formation markedly increased when nitrogen-containing electron-donating substituents were present on the C=N(OH) carbon (compare for instance **IIIb** and **IVa**). Moreover, activities were higher with guanidoximes bearing two N-alkyl substituents than with those having only one N-alkyl (or aryl) substituent (compare **IVa,b** and **c** with **IVd,e** and **f**).

Table 1 : Formation of NO_2^- from incubations of RR'C=N(OH) compounds with liver microsomes from untreated (control) or dexamethasone (DEX)-treated rats in the presence of NADPH[a].

	R	R'	control rats	DEX rats		R	R'	control rats	DEX rats
Ia	Ph	H	< 0.1	3.0	**IIIa**	Ph	NH_2	0.2	2.5
Ib	4-ClPh	H	< 0.1	2.0	**IIIb**	4-ClPh	NH_2	0.7	2.6
Ic	nC_6H_{13}	H	0.2	3.7	**IIIc**	4-($nC_6H_{13}O$)Ph	NH_2	0.6	6.8
IIa	Ph	CH_3	< 0.1	3.2	**IVa**	4-ClPhNH	NH_2	0.6	18.4
IIb	4-ClPh	CH_3	0.1	6.2	**IVb**	4-CF_3PhNH	NH_2	< 0.1	11.0
IIc	nC_5H_{11}	CH_3	< 0.1	7.0	**IVc**		NH_2	< 0.1	28.6
IId	nC_4H_9	C_2H_5	< 0.1	5.1	**IVd**			0.9	62
IIe	-$(CH_2)_5$-		< 0.1	3.0	**IVe**			< 0.1	58
IIf	CH_3CO	CH_3	< 0.1	4.6	**IVf**		NH_2	1.1	79

a) Results are expressed as nmol NO_2^-.(nmol P450)$^{-1}$.(10 min)$^{-1}$ and are means ± 20% from at least four experiments. Incubations were performed as described previously[5] ; they involved 100 µM substrate, 1 µM P450 and 1 mM NADPH in 0.1M phosphate buffer, pH 7.4.

In the case of ketoxime **IIb**, amidoximes **IIIb** and **IIIc**, and guanidoxime **IVa**, we have checked that formation of NO_2^- was accompanied by that of the keto derivative RR'CO that is expected for an oxidative cleavage of the substrate C=N(OH) bond (Table 2). Formation of NO_3^- was also detected ; it was always smaller than that of NO_2^- except in the case of **IIIc**. The amounts of RR'CO were comparable to those of NO_2^- + NO_3^- in all cases studied (Table 2).

Table 2 : Formation of NO_2^-, NO_3^- and RR'C=O from reaction of various RR'C=N(OH) compounds with rat liver microsomes and NADPH.

	R	R'	NO_2^-	NO_3^-	RR'C=O
IIb	4-ClPh	CH_3	6.2	1.2	6.4
IIIb	4-ClPh	NH_2	2.6	1.5	5.1
IIIc	4-($nC_6H_{13}O$)Ph	NH_2	6.8	8.1	12.5
IVa	4-ClPhNH	NH_2	18.4	4.4	17.7

Results expressed as nmol product.(nmol P450)$^{-1}$.(10 min)$^{-1}$ and are means ± 20% of at least four experiments performed with liver microsomes from dexamethasone-treated rats as described in ref.5 in the case of **IIIb**. NO_2^- and NO_3^- measured according to a previously described procedure[14].

The aforementioned data show that 18 different compounds containing a C=N(OH) function act as precursors of nitrogen oxides upon oxidative cleavage of their C=N(OH) bond by rat liver microsomes.

Formation of NO was studied in the case of ketoxime **IIb** and guanidoximes **IVa** and **IVd**. NO formation was followed by detection of P450-and P420-Fe(II)-NO complexes by EPR spectroscopy and found to occur with these three compounds (data not shown) as well as with amidoxime **IIIc**[5]. Thus the formation of nitrogen oxides including NO upon enzymatic oxidation of exogenous compounds involving a C=N(OH) function, which has been previously reported for benzamidoxime **IIIc**[5] and N-hydroxypentamidine[16], appears as a quite general reaction occurring not only for amidoximes and guanidoximes but also for aldoximes and ketoximes. As shown in Table 2, the formation of nitrogen oxides occurs in a manner concomitant to that of the keto compounds RR'CO corresponding to starting RR'C=NOH substrates. This oxidative cleavage of C=N(OH) bonds appears as a new general cytochrome P450-dependent reaction, which is very efficient with cytochromes P450 3A (eq.2). It is particularly fast in the case of N,N'-disubstituted guanidoximes (around 8 turnovers per min). Its mechanism is currently under study ; its significance for the formation of NO from exogenous compounds *in vivo* remains to be established.

$$\textbf{(eq.2)} \quad RR'C{=}N{-}OH \xrightarrow[NADPH + O_2]{P450} RR'C{=}O + NO_2^-(NO_3^-, NO...)$$

R,R' = H, alkyl, aryl, NHR_1, NR_1R_2

References and Notes

1 Moncada, S.; Palmer, R.M.J.; Higgs, E.A. *Pharmacol. Rev.* **1991**, *43*, 109.
2 Knowles, R.G.; Moncada, S. *Biochem. J.* **1994**, *298*, 249.
3 Marletta, M.A. *J. Biol. Chem.* **1993**, *268*, 12231.
4 Boucher, J-L.; Genet, A.; Vadon, S.; Delaforge, M.; Henry, Y.; Mansuy, D. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 880.
5 Andronik-Lion, V.; Boucher, J-L.; Delaforge, M.; Henry, Y.; Mansuy, D. *Biochem. Biophys. Res. Commun.* **1992**, *185*, 452.
6 Clement, B.; Jung, F. *Drug Metab. Dispos.* **1994**, *22*, 486.
7 Compounds **I** and **II**[8], **IIIa** and **IIIb**[17], **IIIc**[18], **IVa**[12], **IVd**[10], **IVe**[10] and **IVf**[19] were described already ; their IR and ^1H NMR data as well as their melting points were in agreement with those previously reported. Compounds **IVb** and **IVc** were completely characterized by IR, ^1H NMR and mass spectroscopy and elemental analysis (C, H, N).
8 Vogel, A.I. *Textbook of Pratical Organic Chemistry*; Longman Scientific & Technical: London, 1989; pp 1332-1370.
9 Nicolaides, D.N.; Varella, E.A. *The Chemistry of Acid Derivatives*; Pataï, S., Ed.; John Wiley & Sons Ltd, 1992, Vol. 2, pp 876-954.
10 Wilkerson, C.J.; Greene, F.D. *J. Org. Chem.* **1975**, *40*, 3112.
11 Reddy, N.L.; Hu, L-Y.; Cotter, R.E.; Fischer, J.B.; Wong, W.J.; McBurney, R.N.; Weber, E.; Holmes, D.L.; Wong, S.T.; Prasad, R.; Keana, F.W. *J. Med. Chem.* **1994**, *37*, 260.
12 Schantl, J.G.; Türk, W. *Sci. Pharm.* **1989**, *57*, 375.
13 Horning, E.C. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, pp 735-736.
14 Green, L.C.; Wagner, D.A.; Glogowski, J.; Skipper, P.L.; Wishnok, J.S.; Tannenbaum, S.R. *Anal. Biochem.* **1982**, *126*, 131.
15 Renaud, J-P.; Boucher, J-L.; Vadon, S.; Delaforge, M.; Mansuy, D. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 53.
16 Clement, B.; Schultze-Mosgau, M-H.; Wohlers, H. *Biochem. Pharmacol.* **1993**, *46*, 2249.
17 Andrewes, C.H.; King, H.; Walker, J. *Proc. R. Soc.* London B **1946**, *133*, 20.
18 Partridge, M.W.; Turner, H.A. *J. Pharm. Pharmacol.* **1953**, *5*, 103.
19 Fukuto, J.M.; Wallace, G.C.; Hszieh, R.; Chaudhuri, G. *Biochem. Pharmacol.* **1992**, *43*, 607.

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