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Oxidation of Arylamidoximes by Hydrogen Peroxide and Horseradish Peroxidase in Water: easy Preparation and X-ray Structure of O-(Arylimidoyl)Arylamidoximes.

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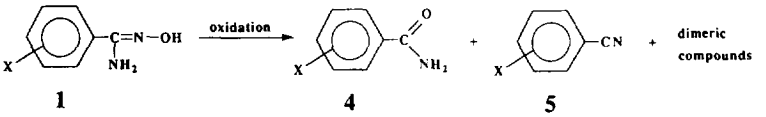
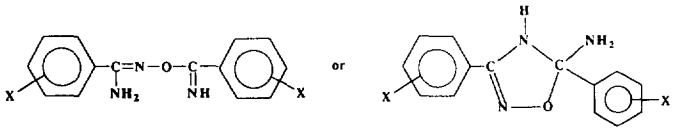
ABSTRACT: The oxidation of arylamidoximes X-C_6H_4-$C(NH_2)$=N-OH (X = H, Me, Cl, NO_2, MeO) by H_2O_2 in the presence of horseradish peroxidase under mild conditions (phosphate buffer pH 7.4, room temperature) yields the corresponding O-(arylimidoyl)arylamidoximes in 30-70% yields. The structure of one of them is established by an X-ray analysis.

Many drugs containing an amidoxime function are biologically active and display antihypertensive, antibacterial, antitrypanocidic, or cytostatic properties.[1] Interest in the oxidation of these compounds recently increased because of the possible formation of nitric oxide (NO), an important biological mediator. Oxidation of arylamidoximes **1** generally leads to a mixture of compounds, including the corresponding amide **4** and nitrile **5** as well as "dimeric" products 2 (or 3) with a formula corresponding to [2 arylamidoximes - NH_2OH].[2-4] Stieglitz first proposed that such a compound obtained upon oxidation of benzamidoxime **1a** (X = H) with potassium ferricyanide exhibited the cyclic structure **3a**.[2] Much more recently, it was proposed, on the basis of IR spectroscopic data, that the compound formed upon oxidation of benzamidoxime **1a** with N-bromo- or N-chlorosuccinimide, which has the same properties as **3a**, had the open-chain structure **2a** of O-(benzimidoyl)benzamidoxime.[5] There is so far no simple method of synthesis of O-(arylimidoyl)arylamidoximes and their existence under the linear form 2 is not definitely established.



1 **4** **5**



2 **3**

In the course of our studies on the oxidation of the arylamidoximes by hemeproteins,[6] we have recently found an easy method of access to O-(arylimidoyl)benzamidoximes upon oxidation of arylamidoximes by hydrogen peroxide (H_2O_2) catalyzed by horseradish peroxidase (HRP)[7] under mild and practical conditions (phosphate buffer pH 7.4, room temperature). The structure of **2c** prepared by this method was clearly established by an X-ray analysis.

Reaction of benzamidoxime **1c**[8] with H_2O_2 and HRP in phosphate buffer led to the appearence of a precipitate which was easily isolated in 57 % yield after centrifugation, filtration and recrystallisation.[9] The corresponding product was found identical to a compound previously prepared, in 30 % yield only, by oxidation of **1c** with N-chlorosuccinimide.[5] Its elemental analysis and IR, ^1H and ^{13}C NMR and mass spectrometry characteristics were in complete agreement with formula **2c**. The X-ray structure of **2c** (Figure 1) definitely establishes the open-chain structure previously proposed.[5,10-11]

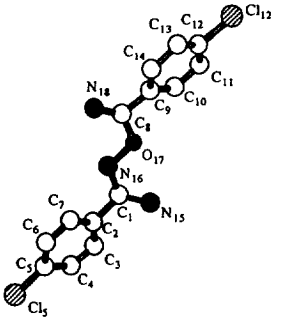
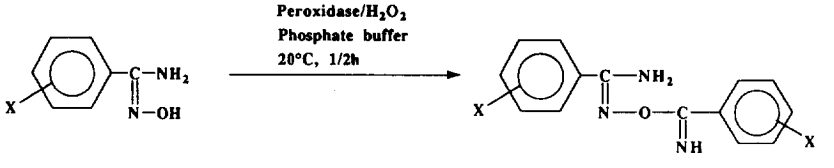


Figure 1: X-Ray structure of **2c**.

Pure compounds **2a-f** were similarly prepared in 30 to 70 % yields from the corresponding arylamidoximes **1a-f**[8] by using the same easy procedure.[9] They show physico-chemical properties similar to those of **2c**.[11]



1a-f **2a-f**

1-2	a	b	c	d	e	f
X	H	4-CH_3	4-Cl	4-NO_2	3-NO_2	4-CH_3O
Yield (%)	27	50	57	61	52	68

Preliminary experiments performed to study the mechanism of HRP-catalyzed oxidation of arylamidoximes **1** showed that such reactions performed under higher dilutions (100 μM) only led to variable amounts of 4-chlorobenzamide **4c** and 4-chlorobenzonitrile **5c**. Increasing the concentration of **1c** led to a progressive increase of the formation of **2c**. Precipitation of **2c** from the reaction mixture greatly facilitated its isolation from starting **1c** and the other products. It is noteworthy that **2c** was not transformed when incubated in the presence of HRP and H_2O_2. However, compounds **2a-f** are unstable in polar solvents and break down in few hours to give the starting arylamidoxime and the corresponding nitrile and amide.

Further studies of the mechanism of this new HRP-catalyzed reaction are under investigation.

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8. Arylamidoximes **1a-f** were prepared from benzonitriles and hydroxylamine hydrochloride (all from Aldrich) according to Tiemann's reaction, and as described more recently.[4,12-13] The identities and purities of **1a-f** were checked by both IR, ^{1}H-NMR and mass spectroscopy, and accord of melting points with literature values: benzamidoxime **1a** m.p.: 77°C (Lit:[4,12] 79-80°C); 4-methylbenzamidoxime **1b** m.p.: 148°C (Lit:[4,14] 145-146°C); 4-chlorobenzamidoxime **1c** m.p.: 134°C (Lit:[4,15] 134-135°C); 4-nitrobenzamidoxime **1d** m.p.: 167°C (Lit:[4,13] 169-170°C); 3-nitrobenzamidoxime **1e** m.p.: 185°C (Lit:[4,13] 186-188°C); 4-methoxybenzamidoxime **1f** m.p.: 121°C (Lit:[16] 122-123°C).

9. *Typical procedure*: O-(4-Chlorobenzimidoyl)4-chlorobenzamidoxime (**2c**):
Arylamidoxime **1c** (43 mg, 0.25 mmol) was dissolved in the minimum amount of 0.1 M HCl (200 ml) using ultrasonic wave, and then added to 0.1 M phosphate buffer pH 7.4 (40 ml) giving an approximate concentration of 6 mM. Horseradish peroxidase (Sigma, 3 mg, about 250 units/mg) dissolved in 2 ml phosphate buffer was added, followed by dropwise addition of H_2O_2 (5 ml, 0.1 M solution) over 5 min at room temperature. A turbidity gradually appeared and the mixture was slowly stirred for further 30 min. The precipitate was separated by centrifugation (3000 rpm, 15 min), washed twice with water, collected by filtration and dried. Pure **2c** was then recrystallised from a mixture of cyclohexane/ethyl acetate (3/1, v/v); yield 22 mg (57 %).

10. *X-ray Analysis of* **2c**: $C_{14}H_{11}N_3OCl_2$. Mw= 308.1. A suitable colorless crystal was investigated on a Siemens P3 diffractometer (Mo Ka radiation = 0.71069 Å, graphite monochromator). Orthorhombic, space group $Pb2_1a$, Z = 4, a = 7.112(4) Å, b = 7.631(4) Å, c = 26.22(1) Å, D_{calc} = 1.43 g.cm^{-3}; reflections up to 2q = 55° of which 1013 with F>3s(F) were kept in refinement calculations. The structure was solved by direct methods using SHELX-76 and refined with SHELX-86[17]. Convergence was reached at R=0.048 and R_w=0.052. Non hydrogen atoms were refined with anisotropic temperature factors; hydrogen atoms were located in

difference 0.18 e.Å^{-3}. Lists of the fractional atomic coordinates, thermal parameters, bond distances and angles are available on request (3 Tables).

11. Melting points were measured on a Kofler melting point apparatus and were uncorrected; Elemental Analyses were performed by Service de Microanalyse, ICSN, Gif sur Yvette , France; ^{1}H NMR spectra were obtained on a Brucker ARX spectrometer operating at 250 MHz: samples were dissolved in CD_3CN and chemical shifts were expressed in ppm relative to TMS; IR spectra were recorded on a Perkin Elmer 783 spectrophotometer after mixing with KBr; Mass Spectra were recorded on a Ribermag R10-10C mass spectrometer operating at 70 eV and using NH_3 in the chemical ionisation mode.

O-(Benzimidoyl)benzamidoxime **2a**: mp 125°C (dec); Anal.: Calc: C: 70.27, H: 5.47, N: 17.56 for $C_{14}H_{13}N_3O$: 239.276, Found: C: 69.95, H: 5.71, N: 16.95; ^{1}H NMR: 8.40 (s, 1H), 8.04 (d, 2H, J = 7.0), 7.74 (d, 2H, J = 8.0), 7.43 (m, 6H), 5.78 (s, 2H); IR: 3480, 3280, 3040, 1640, 1360, 1050, 880, 840; MS: 257 ($M+NH_4^+$, 3), 240 (MH^+, 23), 139 (62), 121 (100).

O-(4-Methylbenzimidoyl)4-methylbenzamidoxime **2b**: mp: 154°C (dec); Anal.: Calc: C: 71.88, H: 6.41, N: 15.72 for $C_{16}H_{17}N_3O$: 267.330, Found: C: 71.70, H: 6.58, N: 15.67; ^{1}H NMR: 8.37 (s, 1H), 8.01 (d, 2H, J = 7.0), 7.71 (d, 2H, J = 8.0), 7.30 (m, 4H), 5.79 (s, 2H), 2.40 (s, 6H); IR: 3470, 3270, 3100, 1630, 1410, 1050, 860, 840; MS: 268 (MH^+, 4), 251 (5), 151 (30), 135 (100).

O-(4-Chlorobenzimidoyl)4-chlorobenzamidoxime **2c**: mp 130°C (dec); Anal.: Calc: C: 54.56, H: 3.60, N: 13.67 for $C_{14}H_{11}N_3OCl_2$: 308.166, Found: C: 54.71, H: 3.89, N: 13.47; ^{1}H NMR: 8.43 (s, 1H), 8.02 (d, 2H, J = 8.3), 7.72 (d, 2H, J = 8.7), 7.42 (d.d., 4H, J = 8.3 and 8.7), 5.84 (s, 2H); IR: 3480, 3280, 3080, 1640, 1400, 1050, 850, 840; MS: 308 (MH^+, 6), 171 (82), 155 (100), 137 (25).

O-(4-Nitrobenzimidoyl)4-nitrobenzamidoxime **2d**: mp 188°C (dec); Anal.: Calc: C: 51.07, H: 3.36, N: 21.27 for $C_{14}H_{11}N_5O_5$: 329.271 Found: C: 51.01, H: 3.49, N: 21.04; ^{1}H NMR: 8.81 (s, 1H), 8.30 (m, 6H), 8.04 (d, 2H, J = 7.5); 6.07 (s, 2H); IR: 3650, 3430, 3400, 3250, 3160, 1640, 1520, 1050, 860; MS: 330 (MH^+, 8), 199 (4), 182 (100), 166 (12).

O-(3-Nitrobenzimidoyl)3-nitrobenzamidoxime **2e**: mp 190°C (dec); Anal.: Calc: C: 51.07, H: 3.36, N: 21.27 for $C_{14}H_{11}N_5O_5$: 329.271, Found: C: 50.99, H: 3.66, N: 21.05; ^{1}H NMR: 8.90 (s, 1H), 8.74 (s, 1H), 8.66 (m, 1H), 8.35 (m, 4H), 7.78 (m, 2H), 7.42 (s, 2H); IR: 3460, 3300, 3260, 3140, 1640, 1530, 1080, 800, 750, 700; MS: 330 (MH^+, 18), 199 (15), 182 (100), 166 (38).

O-(4-Methoxybenzimidoyl)4-methoxybenzamidoxime **2f**: mp 128°C (dec); Anal.: Calc: C: 64.20, H: 5.72, N: 14.04 for $C_{16}H_{17}N_3O_3$: 299.328, Found: C: 63.98, H: 5.88, N: 13.98; ^{1}H NMR: 8.20 (s, 1H), 7.98 (d, 2H, J = 8.8), 7.68 (d, 2H, J = 8.8), 6.93 (m, 4H, J = 8.8), 5.67 (s, 2H), 3.78 (s, 6H); IR: 3480, 3280, 3020, 2960, 1640, 1510, 1250, 1030, 870, 830; MS: 300 (MH^+, 2), 283 (2), 167 (26), 152 (100), 133 (18).

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