A Facile Preparation of Iminoxy Dimers by Hydrogen Peroxide/Peroxidase Oxidation of Aldoximes

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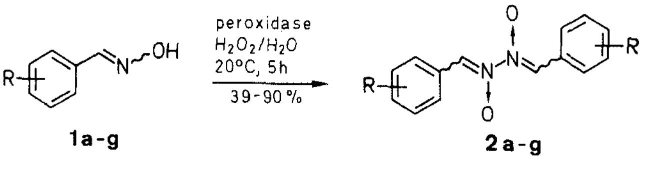
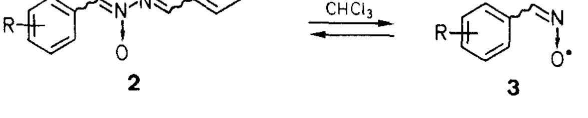
The oxidation of benzaldehyde oximes of the type R-C_6H_4-CH=NOH (R = H, Me, OMe, Cl) by a hydrogen peroxide/horseradish peroxidase system yields the corresponding dimers of iminoxy radicals (benzaldehyde azines) in high yields.

A number of iminoxy radicals have been prepared, using a variety of experimental techniques, for example, oxidation of the oxime compounds by reagents such as lead tetraacetate,[1-3] ceric ammonium nitrate,[4] and silver oxide,[5] hydrogen abstraction from the oximes by photo-induced fragment radicals,[6] and addition of a radical to aromatic nitrile oxides.[6] Iminoxy radicals are considered to be σ radicals, which are characterized by two geometric isomers, *Z* and *E*.[7] As the interconversion between two isomers is slow in the time scale of electron spin resonance (ESR), the radicals are especially suitable for ESR studies on their structures and properties. Although the iminoxy radicals are relatively stable and in equilibrium with the corresponding dimers in an aprotic solvent, they react gradually to form the O–N, O–C, and N–N bonds.[1-3,5] Amongst them, a heterocyclic compound, furoxane, is formed by a 1,3-dipolar cycloaddition reaction from a nitrile oxide.[5]

ESR studies of iminoxy radicals have been carried out by direct generation of the radical in the ESR tube, accompanied by many difficulties owing to their complicated reactions. Recently, ESR study of the iminoxy radical was successfully performed by oxidation of 2-oxyimino-1,3-dioxo compounds with horseradish peroxidase and hydrogen peroxide.[8] Peroxidase is well-known as an enzyme which catalyzes the oxidation, using hydrogen peroxide, as shown in many experiments. It has now been found that the oxidation of substituted benzaldehyde oximes of the type R–C_6H_4–CH=NOH by hydrogen peroxide/horseradish peroxidase yields the corresponding dimers of iminoxy radicals in high yield and these products can be easily separated from the reaction mixtures.



1a-g 2a-g



2 3

1–3	a	b	c	d	e	f	g	h
R	H	4-Me	3-Me	2-Me	4-OMe	4-Cl	2-Cl	4-OH

An aqueous solution of benzaldehyde oxime (**1a**), hydrogen peroxide, and horseradish peroxidase produced a white precipitate. The only product that identified by thin layer-chromatography (TLC) or high-performance liquid chromatography (HPLC) was benzaldehyde azine bis(*N*-oxide) (**2a**). An authentic sample of **2a** was prepared by oxidation of **1a** with lead tetraacetate.[1-3] Similarly, products **2b**, **2e**, **2f** from *para*-substituted benzaldehyde oximes were isolated at the excellent yields, but the dimer **2h** was not obtained in spite of the complete consumption of *para*-hydroxybenzaldehyde oxime (**1h**). The products **2c**, **2d**, **2g** from *meta*-, and *ortho*-substituted benzaldehyde oximes were obtained in moderate yield. It is also demonstrated that hydrogen peroxide/peroxidase oxidation of a mixture of two different benzaldehyde oximes yielded the cross-compound, an unsymmetrical dimer, together with two symmetrical dimers. For example, when *p*-methyl- and *p*-methoxybenzaldehyde oximes (**1b** and **1e**) are mixed in the oxidative solution of hydrogen peroxide/peroxidase, a mixture of **2b**, **2e** and an unsymmetrical dimer was formed in the ratio of 1.0:1.5:2.0, which was determined by HPLC (reverse-phase column, acetonitrile/water, 4:1; t_R = 10, 5.3 and 7.8 min., respectively). The presence of an unsymmetrical dimer was confirmed by ^{1}H-NMR analysis of the mixture.[9] Unfortunately, dimers of diaryl-, alkylaryl-, and dialkyliminoxy radicals were not obtained by the hydrogen peroxide/peroxidase oxidation of the corresponding ketoximes.

The iminoxy dimers are unstable in solution and break down in a few hours to yield several products. However, these dimers are in equilibrium with the iminoxy radicals **3** over a short period of time or under the low temperature. Any dimer in the presence of 2-methyl-2-propanethiol in chloroform was converted to the corresponding benzaldehyde oxime. Further, the formation of iminoxy radicals **3** in the dissociation equilibrium implies that the cross-dimerization of iminoxy radicals occurs when two different dimers are dissolved in chloroform. Actually, an unsymmetrical dimer from an equimolecular mixture of **2b** and **2e** was formed, where the proportion of dimers was in the ratio of 0.6:0.6:0.8 for **2b**, **2e** and the cross dimer at 20°C after 5 hours, respectively.

The benzaldehyde oximes, were prepared from commercially available aldehydes by standard methods. Horseradish peroxidase (102 u/mg) was obtained from Toyobo Company. Before use, each aqueous solution of peroxidase and hydrogen peroxide was adjusted at the arbitrary concentration. The benzaldehyde oxime was dissolved in an excess of water, if necessary, using ultrasonic wave and then the necessary amount of peroxidase was added. The oxidation was started at room temperature by adding dropwise the aqueous solution of hydrogen peroxide. Structures of the products were

Table. Preparation of Iminoxy Dimers **2**

Product	Yield[a] (%)	mp (°C)[b]	Molecular Formula[c] or Lit. mp (°C)	IR (KBr)[d] ν (cm^{-1})	^{1}H-NMR (CDCl$_3$/TMS)[e] δ, J (Hz)	MS (70 eV)[f,g] EI (IB) m/z (%)
2a	87	108–109	104–108[1]	1581, 1451, 1424, 1088, 1069, 844, 759, 685	7.5–8.2 (m, 10H), 8.95 (s, 2H)	238 (M$^+$-2, < 1)
2b	84	123–124	$C_{16}H_{16}N_2O_2$ (268.3)	1580, 1500, 1420, 1290, 1180, 1080, 1060, 850	2.39 (s, 6H), 7.35 (d, 4H, J = 9.3), 8.10 (d, 4H, J = 9.3), 8.95 (s, 2H)	266 (M$^+$-2, < 1)
2c	46	97–98	$C_{16}H_{16}N_2O_2$ (268.3)	1580, 1480, 1440, 1250, 1160, 1070, 910, 780	2.15 (s, 6H), 7.3–8.0 (m, 8H), 8.90 (s, 2H)	266 (M$^+$-2, < 1)
2d	61	102–103	$C_{16}H_{16}N_2O_2$ (268.3)	1580, 1440, 1290, 1090, 870, 830, 760	2.50 (s, 6H), 7.3–8.0 (m, 8H), 9.20 (s, 2H)	266 (M$^+$-2, < 1)
2e	84	134–135	$C_{16}H_{16}N_2O_4$ (300.3)	1601, 1506, 1440, 1254, 1173, 1076, 1027, 851	3.90 (s, 6H), 7.05 (d, 4H, J = 9.9), 8.20 (d, 4H, J = 9.9), 8.95 (s, 2H)	298 (M$^+$-2, < 1)
2f	87	129–131	$C_{14}H_{10}Cl_2N_2O_2$ (309.2)	1588, 1483, 1431, 1088, 841, 761	7.50 (d, 4H, J = 8.8), 8.11 (d, 4H, J = 8.8), 8.89 (s, 2H)	306 (M$^+$-2, < 1)
2g	39	105–106	$C_{14}H_{10}Cl_2N_2O_2$ (309.2)	1580, 1460, 1420, 1090, 860, 820, 750	7.3–8.9 (m, 8H), 9.45 (s, 2H)	306 (M$^+$-2, < 1)

[a] Yield of isolated products **2** based on **1**.

[b] Uncorrected, measured with a Yanagimoto micro-melting point apparatus.

[c] Satisfactory microanalysis obtained: C ±0.29, H ±0.25, N ±0.30.

[d] Recorded on a Hitachi 215 infrared spectrophotometer.

[e] Obtained on a Varian XL-200 spectrometer.

[f] Recorded on a Hitachi M-80B mass spectrometer.

[g] Compound **2a**: MS (70 eV, CI): m/z = 239 (M$^+$-1, <1%).

determined by normal procedures, e.g., IR, ^{1}H-NMR, MS and, elemental analysis and quantitative isolation by HPLC, although their geometry (*E,E*, *E,Z* or *Z,Z* iminoxy dimer) could not be assigned.

Benzaldehyde Azine Bis(*N*-oxide) (Benziminoxy Dimer) (2a); Typical Procedure:

To the solution of **1** (180 mg, 1.5 mmol) and peroxidase (10 mL, 2000 unit) in water (200 mL), is added dropwise H_2O_2 (0.5%, 20 mL)) at r.t. (20 °C) over 1 h and the mixture stirred for 5 h. A milky turbidity gradually appears during the reaction. After separation by an ultracentrifuge, the product is collected by filtration. The pure product is obtained by precipitation from a mixture of $CHCl_3$ and MeOH at −15 °C; yield: 160 mg (87%) (Table).

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(9) Enlargement of ^{1}H-NMR signals (a Varian XL-200, 200 MHz) for the methine protons (δ = 8.92–8.97) showed four peaks, two types of proton for **2b** and **2e**, and two different protons for an unsymmetrical dimer.