# Dehydration of Alkyl- and Arylaldoximes as a New Cytochrome P450-Catalyzed Reaction: Mechanism and Stereochemical Characteristics

Jean-Luc Boucher, Marcel Delaforge, and Daniel Mansuy*

*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400, Université Paris V, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France*

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ABSTRACT: The *Z* isomers of benzaldoxime and 4-(hexyloxy)benzaldoxime were dehydrated into the corresponding nitriles in the presence of rat liver microsomes and NADPH or dithionite. Their *E* isomers remained unchanged under identical conditions. Alkylaldoximes, like phenylacetaldoxime and heptanaldoxime, are also dehydrated under these conditions, the alkylaldoximes being more rapidly transformed than the arylaldoximes. A genetically well-defined P450 expressed in yeast, P450 3A4, the major P450 isozyme in human liver, was also found to be catalytically active for dehydration of (*Z*)-benzaldoxime. All these reactions were found to be catalyzed by P450 Fe(II) as they required the use of intact microsomes in the presence of NADPH or dithionite and were strongly inhibited by $O_2$ and CO as well as by classical P450 inhibitors. A P450 complex characterized by a Soret peak at 442 nm was detected during these reactions; its disappearance was found to be concomitant with the consumption of the aldoxime and the formation of the corresponding nitrile. (*E*)-benzaldoximes and all the studied ketoximes failed to give such complexes with P450 Fe(II). On the basis of these results, a possible mechanism for this new P450 reaction is proposed. It involves a P450 Fe(II) ← N(OH)=CHR complex as a key intermediate and a charge transfer from P450 Fe(II) to the aldoxime C=N bond which results in a cleavage of the aldoxime N–O bond.

Since their discovery about 30 years ago, cytochromes P450 have been found to be ubiquitous hemeproteins in living organisms. Until recently, their catalytic functions appeared to be the transfer of one oxygen from $O_2$ into various substrates, the reduction of some particular substrates (Guengerich and Mac Donald, 1984 ; Ortiz de Montellano, 1986), the isomerization of compounds containing an O–O bond like cyclic peroxides (Haurand & Ullrich, 1985), and the dehydration of certain alkyl hydroperoxides (Song and Brash, 1991). All these reactions involve redox changes of P450 during the catalytic cycle and redox reactions on the substrate. Very recently, a publication has described the dehydration of *n*-butyraldoxime into the corresponding nitrile by rat liver microsomes (De Master et al., 1992). This reaction required only catalytic amounts of NADPH or dithionite and was found to be catalyzed by cytochrome P450 in its ferrous state. This dehydration of *n*-butyraldoxime is a surprising new kind of cytochrome P450 reaction as, at least *a priori*, it is a simple dehydration that occurs on a substrate not containing a peroxidic O–O bond.

In order to determine the mechanism of this reaction, we have studied the interactions of liver cytochromes P450 with various (*E*)- and (*Z*)-aldoximes. This paper reports the corresponding results which show that, in a general manner, cytochromes P450 catalyze the dehydration of alkyl- or arylaldoximes exhibiting a *Z* configuration and which allow us to propose a mechanism for this new P450 reaction.

## MATERIALS AND METHODS

*Physical Measurements.* Absolute and difference UV–vis spectra were performed on a Kontron 941 spectrophotometer. Proton NMR[1] spectra were recorded with a Bruker 250 spectrometer operating at 250 MHz. Samples were dissolved in $CDCl_3$, and chemical shifts are given in ppm relative to tetramethylsilane. *J* values are given in Hz. Infrared spectra were taken on a Perkin-Elmer 783 spectrophotometer.

*Chemicals.* Benzaldehyde, benzonitrile, hexyl bromide, 4-cyanophenol, 4-hydroxybenzaldehyde, phenylacetonitrile, phenylacetaldehyde, heptanal, 4-(hexyloxy)benzoyl chloride, acetophenone, 4-hydroxyacetophenone, 2-heptanone, 3-heptanone, 4-chlorobenzaldehyde, 4-chloroacetophenone, 4-chlorobenzamide, and hydroxylamine hydrochloride were purchased from Aldrich. All other solvents and chemicals were of the highest purity commercially available.

*Preparation of Aldoximes.* Mixtures of (*Z*)- and (*E*)-aldoximes were obtained by conventional methods from aldehydes and hydroxylamine hydrochloride in aqueous ethanol as described previously (Vogel, 1989). Pure (*Z*)-benzaldoxime and (*Z*)-4-(hexyloxy)benzaldoxime were obtained as reported previously from the mixture of (*Z*)- and (*E*)-aldoximes: they were first converted into a hydrochloride on treatment with anhydrous hydrogen chloride and then neutralized in a rapid sequence of dissolution in excess sodium hydroxide, reacidification with ammonium chloride, and extraction with diethyl ether (Schoenewaldt et al., 1968).

*Benzaldoxime* was prepared by reacting 50 mmol of redistilled benzaldehyde with 150 mmol of hydroxylamine hydrochloride and 200 mmol of sodium acetate in 30 mL of a water/ethanol mixture (2/1) for 2 h at room temperature. $^1H$ NMR spectra of the distilled product (39 mmol, 78%), $Eb_{20}$, 122 °C (lit. (Luxmore, 1896) $Eb_{12}$, 118 °C), showed

[1] Abbreviations : GC, gas chromatography; HPLC, high-pressure liquid chromatography; NMR, nuclear magnetic resonance; PB, phenobarbital; RP-HPLC, reversed-phase high-pressure liquid chromatography.

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that a mixture of *E* (90%) and *Z* (10%) isomers was obtained. $^1$H NMR: **2*E*** 8.23 (s, 1H, –CH=N–), 7.6 (m, 2H), and 7.42 (m, 3H); **2*Z*** 7.93 (m, 2H), 7.43 (m, 3H), and 7.38 (s, 1H, CH=N). Pure *(E)-benzaldoxime*, **2*E***, was obtained after chromatography on silica using a mixture of dichloromethane/methanol (99/1) as solvent: *E* isomer, $R_f$ 0.55, and *Z* isomer, $R_f$ 0.25. Pure *(Z)-benzaldoxime*, **2*Z***, was obtained through isomerization of the distilled mixture of (*E*)- and (*Z*)-benzaldoximes. A solution of 5 mmol of (*E*)- and (*Z*)-benzaldoxime in 5 mL of dry benzene was heated to boiling, and a flow of anhydrous hydrogen chloride gas was sparged through the vigorously stirred hot solution for about 30 min. After addition of 5 mL of hexane and cooling to room temperature, the resulting crystalline hydrochloride was filtered and washed with two 5-mL portions of dry *n*-hexane. No attempt was made to isolate this intermediate. Diethyl ether (10 mL) and an aqueous solution of sodium hydroxide (5 mL, 1 M) were mixed and cooled to 10 °C with stirring, and the hydrochloride was rapidly added. As soon as all the solid has dissolved, a solution of 2.0 g of ammonium chloride in 5 mL water was added. The layers were then separated, and the aqueous layer was reextracted with diethyl ether. The combined ethereal extracts were dried, filtered, and evaporated under vacuum to a thick slurry. Pure (*Z*)-benzaldoxime was filtered, washed with *n*-hexane, and air-dried to constant weight (4.4 mmol, 88% yield, mp 129 °C (lit. (Schoenewaldt et al., 1968) mp 130 °C). Its $^1$H NMR characteristics were found to be identical to those previously described.

*Phenylacetaldoxime* was prepared by reacting 10 mmol of phenylacetaldehyde dissolved in 10 mL of ethanol with 20 mmol of hydroxylamine hydrochloride and 20 mmol of sodium hydroxide (5 mL of a 4 N solution) for 2 h at room temperature. Usual workup gave 5.5 mmol (55% of theory) of a product recrystallized from ethanol, mp 102 °C, (lit. (Vogel, 1989) mp 99 °C). $^1$H NMR spectra showed that a mixture of *Z* (75%) and *E* (25%) isomers was obtained: 8.84 (s, broad, 0.75H), 8.25 (s, broad, 0.25H), 7.52 (t, 0.25H, $J$ = 6.2, (*E*)-CH=N), 7.28 (m, 5H), 6.89 (t, 0.75H, $J$ = 4.8, (*Z*)-CH=N–), 3.73 (d, 1.5H, $J$ = 4.8), and 3.52 (d, 0.5H, $J$ = 6.2).

*Heptanaldoxime* was prepared as previously described from heptanal. Recrystallization from pentane gave a 43% yield of pure heptanaldoxime, mp 58 °C, (lit. (Vogel, 1989) mp 57 °C). Its $^1$H NMR spectrum indicated that a mixture of *Z* (45%) and *E* (55%) isomers was obtained: 8.30 (s, broad, 1H), 7.40 (t, 0.55H, $J$ = 7.0), 6.69 (t, 0.45H, $J$ = 6.2), 2.35 (dt, 0.9H, $J$ = 6.2), 2.16 (dt, 1.1H, $J$ = 7.0), 1.45 (m, 2H), 1.30 (m, 6H), and 0.85 (m, 3H).

*4-(Hexyloxy)benzaldehyde*, **1b**, was prepared by alkylation of the sodium salt of 4-hydroxybenzaldehyde with hexyl bromide. A dissolution of 30 mmol of Na in 20 mL of dry ethanol was performed, and 30 mmol of 4-hydroxybenzaldehyde was added. The solution was stirred for 15 min at room temperature before the addition of 40 mmol of hexyl bromide and under reflux for 6 h. Column chromatography of the crude mixture afforded 23.4 mmol (78% yield) of pure 4-(hexyloxy)benzaldehyde, $R_f$ 0.70, silica, ethyl acetate/cyclohexane = 1/3. $^1$H NMR: 9.80 (s, 1H), 7.75 (d, 2H, $J$ = 8.2), 6.92 (d, 2H, $J$ =8.2), 3.96 (t, 2H, $J$ = 5.8), 1.75 (m, 2H), 1.28 (m, 6H), and 0.85 (t, 3H, $J$ = 6.2). IR (film): 2930, 2720, 1690, 1600, 1250, 1160, and 830 cm$^{-1}$.

*4-(Hexyloxy)benzaldoxime*, **1**, was obtained as a mixture of *Z* (6%) and *E* (94%) isomers by reacting 2 mmol of 4-(hexyloxy)benzaldehyde dissolved in 10 mL of ethanol with 4 mmol of hydroxylamine hydrochloride and 4 mmol of sodium hydroxide (2 mL of 2 M solution) for 2 h at 60 °C. Usual workup and recrystallization from ethanol gave 1.04 mmol (52% of theory) of 4-(hexyloxy)benzaldoxime, mp 48 °C. $^1$H NMR: 8.06 (s, 0.94H), 7.87 (d, 0.12H, $J$ = 8.8), 7.48 (d, 1.88H, $J$ = 8.5), 7.25 (s, 0.06H), 6.87 (d, 2H, $J$ = 8.5), 3.96 (t, 2H, $J$ = 6.8), 1.78 (m, 2H), 1.30 (m, 6H), 0.87 (t, 3H, $J$ = 6.5). IR (KBr): 3250 (broad), 3040, 2920, 1610, 1520, 1250, 1170, and 830 cm$^{-1}$. Mass spectrum (EI, *m/z*): 221 ($M^+$), 205, 203, 121 (100). Pure (*Z*)-4-(hexyloxy)benzaldoxime was obtained after isomerization of the mixture of *E* and *Z* isomers as described for the preparation of pure (*Z*)-benzaldoxime and recrystallization from ethanol, yield 75%, mp 104 °C. $^1$H NMR: 7.88 (d, 2H, $J$ = 8.8), 7.25 (s, 1H), 6.88 (d, 2H, $J$ = 8.8), 3.96 (t, 2H, $J$ = 6.8), 1.78 (m, 2H), 1.30 (m, 6H), and 0.88 (t, 3H, $J$ = 6.5).

*4-Chlorobenzaldoxime* was prepared as previously described from 4-chlorobenzaldehyde. Recristallization from ethanol gave a 68% yield of pure 4-chlorobenzaldoxime, mp 109 °C (lit. (Vogel, 1989) mp 107 °C). Its $^1$H NMR spectrum indicated that a mixture of *Z* (5%) and *E* (95%) isomers was obtained. *Z* isomer: 7.57 (s, 1H), 7.97 (d, 2H, $J$ = 8.5), and 7.47 (d, 2H, $J$ = 8.5). *E* isomer: 8.13 (s, 1H), 7.59 (d, 2H, $J$ = 8.5), and 7.43 (d, 2H, $J$ = 8.5).

*Preparation of Ketoximes.* Ketoximes were obtained by conventional methods from ketones and hydroxylamine hydrochloride in aqueous ethanol as described previously (Vogel, 1989).

*Preparation of Nitriles. Heptanenitrile* was obtained by reaction of sodium cyanide (0.08 mol) with hexyl bromide (0.06 mol) dissolved in 50 mL of dry methanol. After the mixture was heated for 15 h at reflux and conventional workup, pure heptanenitrile was obtained in 83% yield. $Eb_{13}$ 73 °C (lit. (Vogel, 1989) $Eb_{10}$ 70 °C). $^1$H NMR: 2.29 (t, 2H, $J$ = 6.6), 1.62 (m, 2H), 1.37 (m, 2H), 1.27 (m, 4H), and 0.85 (t, 3H, $J$ = 6.9). IR (film): 2240 cm$^{-1}$.

*4-(Hexyloxy)benzonitrile*, **1a**, was prepared by alkylation of the sodium salt of 4-cyanophenol with hexyl bromide. A dissolution of 30 mmol of Na in 20 mL of dry ethanol was performed, and 30 mmol of 4-cyanophenol was added. The solution was stirred for 15 min at room temperature. Hexyl bromide (40 mmol) was added, and the mixture was stirred under reflux for 15 h. Recrystallization from ethanol/water = 9/1 afforded 24.9 nmol (83% yield) of pure 4-(hexyloxy)-benzonitrile, mp 28 °C. $^1$H NMR: 7.53 (d, 2H, $J$ = 8.7), 6.90 (d, 2H, $J$ = 8.7), 3.98 (t, 2H, $J$ = 6.5), 1.72 (m, 2H), 1.25 (m, 6H), and 0.82 (t, 3H, $J$ = 6.7). IR (KBr): 3040, 2920, 2220, 1610, 1510, 1210, 1170, and 830 cm$^{-1}$. Mass spectrum (EI) *m/z* = 203 ($M^+$).

*Preparation of Rat Liver and Yeast Microsomes.* Male Sprague–Dawley rats were provided laboratory chow and water ad libitum. After 10 days of adaptation, animals were treated with phenobarbital (80 mg/kg in 0.9% saline, 20 mg/mL, i.p. for 4 days), and liver microsomes were prepared by differential centrifugation (Kremers et al., 1981) and stored at –80 °C. Microsomes from the W(R) yeast strain expressing P450 NF25 were prepared as described previously (Peyronneau et al., 1992; 1993). Protein concentrations were determined by the method of Lowry (Lowry et al., 1951) with bovine serum albumin as standard. Cytochrome P450 contents were determined by the method of Omura and Sato (Omura and Sato, 1964).

*Spectral Measurements.* A 1 μM solution of P450 from rat liver microsomes in deaerated 0.1 M phosphate buffer (pH 7.4) was equally divided between two deaerated cuvettes, and a slow stream of argon was passed at the surface of the solution

during at least 20 min. A base line was recorded; the studied substrate was added to the sample cuvette, and NADPH (or $Na_2S_2O_4$) was then added to both cuvettes. UV–vis spectra were recorded between 380 and 500 nm at room temperature. In some experiments, aliquots (50 μL) of the sample cuvette were taken after different incubation times (0–50 min), treated with 50 μL of $CH_3CN$ containing 20 μM internal standard (see below, in the paragraph HPLC analysis), and centrifuged, and the supernatant was analyzed by reversed-phase HPLC (RP-HPLC).

*Incubation Procedures.* All incubations were performed under anaerobic conditions, using argon-degassed phosphate buffer pH 7.4. A typical incubation procedure is as follows: to a suspension of rat liver microsomes (or P450 NF25 containing yeast) (1 μM P450) was added NADPH or $Na_2S_2O_4$ (final concentration: 1 or 10 mM, respectively). After a preincubation time of 1 min at 37 °C, substrate (usual concentration 100 μM) was added and incubations were performed for the indicated times. Incubations were stopped by the addition of an equal volume of cold $CH_3CN$ containing 20 μM internal standard and centrifuged for 30 min at 3000 rpm at 4 °C, and the supernatants were analyzed by RP-HPLC. When heptanaldoxime or phenylacetaldoxime was used as substrate, the incubations were stopped by the addition of 20% (v/v) cold $CH_3CN$ and extracted twice with ether. After careful evaporation of ether under a flow of nitrogen, the products were redissolved in pentane and analyzed by GC, identified by comparison with authentic samples, and quantified using *n*-dodecane as a standard.

*HPLC Analysis.* HPLC analyses were performed on a Nucleosil C18 column (Shandon, SFCC) (5 μm, 250 × 4.6 mm) with a Kontron analytical HPLC system, a Beckman D165 variable dual-wavelength detector, and a D2000 Merck integrator.

(*Z*)- and (*E*)-benzaldoximes, benzaldehyde, and benzonitrile (retention times of 9.4, 10.2, 12.6, and 15.4 min, respectively) were separated using a 65/35 (v/v) mixture of water containing 5 mM $H_3PO_4$ and $CH_3CN$ as eluent. The flow rate was 1 mL/min, and detection was performed at 230 nm. Quantitations were done using 4-chlorobenzamide (retention time 7.1 min) as a standard.

(*Z*)- and (*E*)-4-(hexyloxy)benzaldoxime (retention times 14.5 min and 13.4 min, respectively), 4-(hexyloxy)benzaldehyde, and 4-(hexyloxy)benzonitrile (retention times 19.5 min and 21.3 min, respectively) were separated using a linear gradient from 60% $CH_3CN$ in $H_2O$ containing 5 mM $H_3PO_4$ to 75% $CH_3CN$ in 30 min. Quantitations were done using 4-(hexyloxy)benzamide (retention time 8.8 min) as a standard. The flow rate was 1 mL/min, and detection was done at 254 nm.

*GC Analysis.* Gas chromatography (GC) analyses were performed on an Intersmat IGC 120 FL apparatus with a FFAP 5% column. The temperature of the oven was increased from 100 to 220 °C at 6 °C/min to separate heptanenitrile, *n*-heptanal, and *n*-dodecane and from 120 to 240 °C at 10 °C/min to separate phenylacetonitrile, phenylacetaldehyde, and *n*-dodecane.

## RESULTS

*Dehydration of 4-(Hexyloxy)benzaldoxime by Liver Microsomes.* Anaerobic incubations of 100 μM 4-(hexyloxy)-benzaldoxime, **1**, which existed as a 20:80 mixture of *E* and *Z* isomers, in the presence of liver microsomes (1 μM P450) from phenobarbital (PB)-treated rats containing NADPH or dithionite were analyzed by reversed-phase HPLC. In addition to the starting aldoxime, two new compounds absorbing at 254 nm were observed. The major product had a retention time identical to that of 4-(hexyloxy)benzonitrile, **1a**, whereas the minor product was coeluted with 4-(hexyloxy)benzaldehyde, **1b**. After similar incubations performed on much larger amounts of **1**, the final organic products were extracted with $CH_2Cl_2$ and submitted to spectroscopic studies. The $^1H$ NMR spectrum of the reaction mixture exhibited a doublet at 7.53 ppm (in $CDCl_3$) which is characteristic of the aromatic protons of nitrile **1a** in the position ortho to the CN group. Its IR spectrum exhibited an intense peak at 2230 $cm^{-1}$ characteristic of a CN group. Finally, thin-layer chromatography analysis of this mixture also revealed the presence of a major product having a $R_f$ identical to that of nitrile **1a**. All these results clearly established the formation of **1a** as a major metabolite of **1**.

Table 1: Reaction of 4-(Hexyloxy)benzaldoxime, **1**, with Rat Liver Microsomes in the Presence of NADPH under Anaerobic Conditions[a]

| conditions | products: nitrile **1a** (μM) (% of CS) | products: aldehyde **1b** (μM) |
|---|---|---|
| complete systems (CS) | 24.0 (100) | 1.0 |
| boiled microsomes | 1.4 (6) | 0.8 |
| – microsomes | 0 (0) | 0 |
| – NADPH | 1.0 (4) | 0.2 |
| – NADPH + NADH | 6.2 (26) | 0.2 |
| + NADH | 25.8 (106) | 1.0 |
| + CO | 2.0 (8) | 0.2 |
| + miconazole (100 μM) | 7.8 (32) | 0.2 |
| – NADPH + $Na_2S_2O_4$ | 31.4 (130) | 1.6 |
| – microsomes + $Na_2S_2O_4$ | 0 (0) | 1.2 |
| + $O_2$ | 2.4 (10) | 3.4 |

[a] The complete system consists in liver microsomes from PB-treated rats (1 μM P450; 3.7 nmol of P450 per mg of protein) in anaerobic 0.1 M phosphate buffer, pH 7.4, containing 1 mM NADPH and 100 μM aldoxime **1** (mixture of *E* and *Z* isomers 20:80). Products were determined after 20 min reaction at 37 °C. Results are means ±15% from three independent experiments.

Table 1 shows the effects of various factors on the formation of **1a** upon the microsomal metabolism of **1**. Formation of **1a** was an enzymatic reaction as boiled microsomes were completely inactive. The presence of a reducing agent, NADPH or dithionite, was also required. The use of NADH instead of NADPH gave a much lower activity (26%). The presence of $O_2$ in the incubate greatly inhibited the formation of **1a**, as only 10% of the activity remained in aerobic incubations. Finally, classical P450 inhibitors like miconazole and CO strongly decreased the formation of **1a** (68 and 92% inhibition, respectively) (Table 1). Aldehyde **1b** was always formed as a very minor product in all anaerobic incubations and slightly increased in the presence of $O_2$.

These data indicate that the dehydration of **1** into **1a** was catalyzed by a P450 enzyme in its reduced state. An identical conclusion has been previously drawn from similar studies performed on *n*-butyraldoxime (De Master et al., 1992). Thus, it seems that P450 enzymes are able to catalyze the dehydration of aryl- as well as alkylaldoximes.

In order to determine the role of the stereochemistry of aldoximes in that reaction, we have prepared pure *E* and *Z* stereoisomers of benzaldoxime and studied their fate in the presence of liver microsomes.

*Reaction of E- and Z-Benzaldoximes with Liver Microsomes in the Presence of NADPH.* Incubation of a 1:1 mixture of (*Z*)- and (*E*)-benzaldoximes, **2Z** and **2E**, with microsomes from PB-treated rats in the presence of NADPH

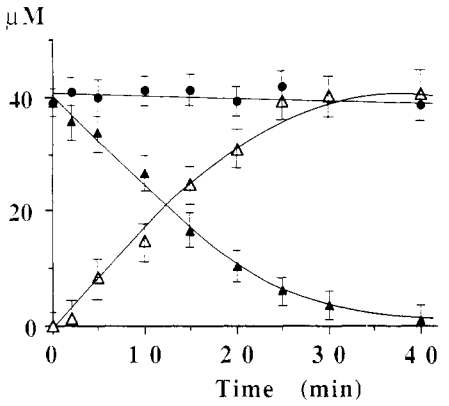


FIGURE 1: Fate of a mixture of (*Z*)- and (*E*)-benzaldoximes incubated with anaerobic rat liver microsomes in the presence of dithionite, as a function of time: ▲, disappearance of **2*Z***; ●, fate of **2*E***; △, formation of benzonitrile. Liver microsomes from PB-treated rats (2 μM P450; 2.6 nmol P450 per mg protein) in anaerobic 0.1 M phosphate buffer pH 7.4 containing 10 mM sodium dithionite and 40 μM (*Z*)-benzaldoxime and 41 μM (*E*)-benzaldoxime were incubated at 37 °C.

Table 2: Dehydration of Benzaldoxime **2** Catalyzed by Rat Liver Microsomes in the Presence of NADPH[a]

| conditions | benzonitrile (μM) (% of CS + **2*Z***) |
|---|---|
| complete system (CS) + **2*Z*** | 24 (100) |
| CS + **2*Z*** – NADPH + $Na_2S_2O_4$ (10 mM) | 20 (84) |
| CS + **2*E*** | <1 (<4) |
| CS + **2*Z*** but boiled microsomes | 1.2 (5) |
| CS + **2*Z*** – microsomes | <0.5 (0) |
| CS + **2*Z*** – NADPH | 0.7 (2) |
| CS + **2*Z*** – NADPH + NADH (1 mM) | 4.3 (18) |
| CS + **2*Z*** + NADH | 27.0 (108) |
| CS + **2*Z*** + miconazole (20 μM) | 1.9 (8) |
| CS + **2*Z*** + CO | <0.5 (0) |
| CS + **2*Z*** + $O_2$ | <0.5 (0) |

[a] Conditions identical to those of Table 1 (1 μM P450; 2.6 nmol P450 per mg protein). CS is for the complete system involving anaerobic intact microsomes and 1 mM NADPH. Concentration of **2*Z*** or **2*E*** was 100 μM. Mean values ±15% from three independent experiments.

under anaerobic conditions led to the formation of benzonitrile, **2a**, which was characterized by its HPLC retention time and by its $^1H$ NMR and IR spectra after extraction by $CH_2Cl_2$. Figure 1 reports the fate of a mixture of **2*Z*** and **2*E*** and the formation of **2a** as a function of time. It clearly shows that (*Z*)-benzaldoxime progressively disappeared with concomitant formation of the corresponding nitrile whereas (*E*)-benzaldoxime remained intact during the reaction. Table 2 completely confirmed this result, as 24 nmol of benzonitrile was formed per nmol P450 in 20 min from anaerobic incubation of **2*Z*** (100 μM) with liver microsomes (1 μM P450) in the presence of NADPH, whereas only very low amounts of nitrile (at the level of detection limit) could be detected under identical conditions but with **2*E*** as substrate. Under these conditions, formation of **2a** from **2*Z*** was linear as a function of time for at least 20 min and was linearly dependent on P450 concentration (up to 2 μM) (data not shown). As previously found in the case of 4-(hexyloxy)benzaldoxime, intact liver microsomes and NADPH (or dithionite) were required for dehydration of **2*Z*** into **2a**, and the use of NADH instead of NADPH gave a much lower activity. Formation of **2a** was completely inhibited under normal aerobic conditions or in the presence of carbon monoxide (Table 2). Another classical P450 inhibitor, miconazole, led to a 92% decrease of the activity, even at a relatively low concentration (20 μM).

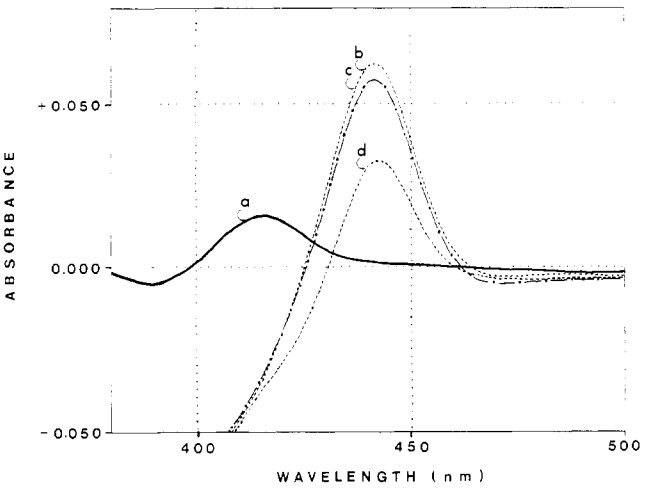


FIGURE 2: Difference spectra of rat liver microsomes in the presence of (*Z*)-benzaldoxime with or without dithionite. Both cuvettes contained a suspension of liver microsomes from PB-treated rats (1 μM P450, 2.0 nmol of P450 per mg protein) in 0.1 M phosphate buffer pH 7.4. (a) Addition of 100 μM (*Z*)-benzaldoxime to the sample cuvette. Then, addition of 10 mM sodium dithionite to both cuvettes and recording of the spectrum after 30 s (b), 5 min (c), and 20 min (d).

These characteristics of the microsomal dehydration of benzaldoxime, i.e., requirement of P450 Fe(II) formed in anaerobic microsomes in the presence of NADPH (or dithionite) and strong inhibition by $O_2$ and CO, are identical to those observed during microsomal dehydration of *n*-butyraldoxime (De Master et al., 1992) and 4-(hexyloxy)-benzaldoxime (Table 1). They also establish a strict stereochemical requirement of the dehydration reaction as only the (*Z*)-benzaldoxime isomer is dehydrated (Figure 1 and Table 2). Experiments similar to those reported in Figure 1 but performed on a 80:20 mixture of the *Z* and *E* isomers of **1** led to an identical conclusion about the exclusive dehydration of the *Z* isomer (data not shown).

*Spectral Interaction of Various Aldoximes and Ketoximes with Microsomal P450s.* Addition of increasing concentrations of **2*Z*** to an anaerobic suspension of liver microsomes from PB-treated rats only gave a weak difference spectrum characterized by a peak at 414 nm and a trough at 393 nm. This so-called reverse type I spectrum should correspond to a shift of cytochrome P450 Fe(III) originally in the high-spin state to the low-spin state (Dawson & Sono, 1987). Then, addition of NADPH to both cuvettes led to a fast appearance of an intense difference spectrum exhibiting a peak at 442 nm and a trough around 420 nm. Identical difference spectra were observed when **2*Z*** was added to microsomal suspensions reduced by dithionite (10 mM) (Figure 2). Under these conditions, the 442-nm absorbing complex immediately appeared after **2*Z*** addition. This complex slowly disappeared as a function of time, its rate of disappearence depending upon the starting concentration of **2*Z***. Figure 3 shows a typical experiment in which addition of 20 μM **2*Z*** to an anaerobic suspension of PB-treated rat liver microsomes containing 1 mM NADPH led to a maximum formation of the 442-nm absorbing complex within 2 min. Then, this complex progressively disappeared in about 30 min. RP-HPLC analysis of the corresponding mixture showed a simultaneous consumption of **2*Z*** and a concomitant appearance of **2a**, **2*Z*** being completely dehydrated into **2a** after 30 min.

Under identical conditions, **2*E*** was found to be able to give a weak reverse type I spectrum with PB-treated rat liver microsomes which was almost identical to that found with

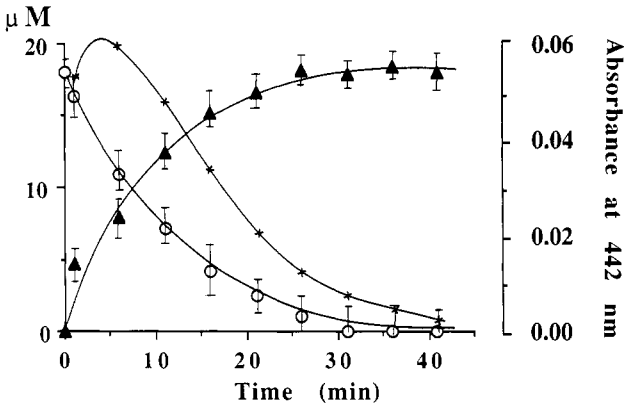


FIGURE 3: Dehydration of (*Z*)-benzaldoxime by anaerobic rat liver microsomes in the presence of NADPH followed by HPLC and difference visible spectroscopy: ○, disappearance of **2*Z***; ▲, formation of benzonitrile; *, fate of the 442-nm absorbing complex. Liver microsomes from PB-treated rats (2 μM P450; 2.6 nmol P450 per mg protein) in anaerobic 0.1 M phosphate buffer pH 7.4 containing 1 mM NADPH and 20 μM **2*Z*** were incubated in a 1-mL spectrophotometric cuvette at ambient temperature. The reaction was followed by difference visible spectroscopy (the reference cuvette containing the same medium without **2*Z***) and HPLC as described in the Materials and Methods.

**2*Z***. However, contrary to **2*Z***, its isomer **2*E*** completely failed to lead to a 442-nm difference spectrum when added to anaerobic microsomes in the presence of NADPH or dithionite under conditions identical to those of Figure 3 (data not shown).

These results establish a clear relationship between the aptitude of aldoximes to form a 442-nm absorbing P450 complex and their ability to be dehydrated to the corresponding nitrile. It is thus tempting to propose that this complex could be an intermediate in nitrile formation.

It was previously shown that butyraldoxime also interacted with rat liver microsomes in the presence of dithionite with formation of a 442-nm absorbing complex (De Master et al., 1992). The authors did not mention the stereochemistry of the sample of aldoxime that they have used, but it is likely that butyraldoxime existed as a mixture of *Z* and *E* isomers. In order to further confirm the generality of the formation of P450 complexes with aldoximes, we have studied the spectral interaction of a series of aryl- and alkylaldoximes and ketoximes with microsomal cytochromes P450. Table 3 shows that all the aldoximes tested that contained a *Z* isomer, namely 4-(hexyloxy)benzaldoxime, 4-chlorobenzaldoxime, heptanaldoxime, and phenylacetaldoxime exhibit a spectral behavior identical to that of (*Z*)-benzaldoxime. They all gave weak reverse type I difference spectra with microsomes containing P450 in its resting ferric state. The intensity of these reverse type I spectra ($\Delta A$(415–390 nm) per μM P450) was between 0.004 and 0.018 for 100 μM aldoxime (Table 3) and was maximum ($\Delta A \approx 0.02$) for 1 mM aldoxime (data not shown). Moreover, all the aldoximes studied led to intense difference spectra characterized by a Soret peak at 442 nm with microsomes treated by dithionite or NADPH under anaerobic conditions. These difference spectra appeared within 1 min after addition of aldoximes and then disappeared with a rate which was dependent on the concentration and nature of the aldoxime *Z* isomer. Maximum intensities of these complexes were obtained for aldoxime concentrations between 100 μM and 1 mM and corresponded to $\Delta A$ (442–490 nm) per μM P450 around 0.065. The $\epsilon$ values for the spectra of these complexes are not known; however, the $\epsilon$ values of P450 Fe(II)–ligand complexes generally vary between 0.05 and 0.09 ($\Delta A(\lambda_{max}$-490 nm) $\mu M^{-1}$ $cm^{-1}$) (Dawson & Sono, 1987). Thus, a great part of microsomal P450s appear to be involved in the formation of 442-nm absorbing complexes with aldoximes. As far as the nature of these complexes is concerned, one should note that the disappearance of the 442-nm peak upon CO addition and its replacement by a 450-nm peak, whose intensity corresponds to the total starting P450 (data not shown), shows that the 442-nm peak is not derived from any type of P450 denaturation and that the exogenous ligand of the 442-nm absorbing complex is replaced by CO. It is noteworthy that the nitriles RCN corresponding to aldoximes RCH=NOH were unable to give 442-nm spectra when added to dithionite-reduced microsomes. Moreover, contrary to (*Z*)-benzaldoxime, **2*Z***, benzylamine, which could have been formed by reduction of **2*Z***, failed to give any difference spectrum with dithionite-reduced microsomes under identical conditions (100 μM compound and 1 μM P450). The more hydrophobic amine which could have been formed upon reduction of aldoxime **1**, 4-(hexyloxy)benzylamine, led to a difference spectrum with dithionite-reduced microsomes characterized by two peaks at 428 and 447 nm very different and much weaker than the one observed with aldoxime **1** (data not shown).

Contrary to aldoximes, ketoximes bearing similar substituents and derived from various para-substituted acetophenones or from heptan-2-one and heptan-3-one completely failed to give such a 442-nm absorbing complex with dithionite-reduced microsomes although they gave classical type I ($\lambda_{max} \approx 390$ nm and $\lambda_{min} \approx 420$ nm) difference spectra with cytochrome P450 Fe(III) (Table 3).

*Dehydration of Various Aldoximes by Anaerobic Rat Liver Microsomes in the Presence of Dithionite.* All the aldoximes (mixtures of *E* and *Z* isomers) which were found to give 442-nm-absorbing complexes (Table 3) were also found to undergo a P450 Fe(II)-catalyzed dehydration, as (*Z*)-benzaldoxime (Figure 1) and butyraldoxime (De Master et al., 1992). Formation of heptanenitrile and phenylacetonitrile after reaction of heptanaldoxime and phenylacetaldoxime with rat liver microsomes and dithionite was established by comparison of the $^1H$ NMR spectra of the reaction mixture with those of authentic samples. It was confirmed by the identical retention times of the metabolites and authentic nitriles in gas chromatography (GC). Table 4 shows a comparison of the initial rates of dehydration of several aldoximes in the presence of rat liver microsomes and dithionite under identical conditions. Arylaldoximes **1** and **2** gave rates markedly lower than the two studied alkylaldoximes, heptanaldoxime, and phenylacetaldoxime.

*Dehydration of (Z)-Benzaldoxime Catalyzed by P450 3A4 Expressed in Yeast.* In order to confirm that a well-defined cytochrome P450 was able to catalyze the dehydration of aldoximes, we have studied the reaction of (*E*)- and (*Z*)-benzaldoxime with microsomes from yeast expressing the cDNA of P450 NF25, a member of the P450 3A subfamily which is the major P450 of human liver (Guengerich and Turvy, 1991). Microsomes from the NF25-W(R) yeast strain, which overexpresses yeast cytochrome P450 reductase and produces P450 NF25, were used (Peyronneau et al., 1992). As shown in Table 5, (*E*)-benzaldoxime was not dehydrated by these microsomes in the presence of dithionite under anaerobic conditions. On the contrary, (*Z*)-benzaldoxime was dehydrated into benzonitrile under identical conditions (eight turnovers in 30 min). This reaction was slighty faster in the presence of added cytochrome $b_5$ (purified from rabbit liver, 1 equiv per P450). This is in agreement with the beneficial effect of cytochrome $b_5$ on several activities of P450 NF25

Table 3: Spectroscopic Studies on the Interaction of Aldoximes and Ketoximes with Microsomal Cytochromes P450

| oxime $R_1R_2C$=NOH | difference spectra[a] with P450 Fe(III) $\lambda_{max}$ | P450 Fe(III) $\lambda_{min}$ | P450 Fe(III) $\Delta A$ ($\lambda_{max} - \lambda_{min}$) | P450 Fe(II) $\lambda_{max}$ | P450 Fe(II) $\Delta A$ (442–490 nm) |
|---|---|---|---|---|---|
| $R_1$ = Ph, $R_2$ = H (*E*:*Z* = 50:50) | 415 | 393 | 0.024 | 442 | 0.055 |
| *Z* isomer[b] | 414 | 393 | 0.003 | 442 | 0.030 |
| *E* isomer[b] | 417 | 394 | 0.003 | nd | |
| $R_1$ = Ph, $R_2$ = $CH_3$ | nd[c] | nd | | nd | |
| $R_1$ = 4-ClPh, $R_2$ = H (*E*:*Z* = 95:5) | 412 | 391 | 0.004 | 442 | 0.020 |
| $R_1$ = 4-ClPh, $R_2$ = $CH_3$ | 391 | 425 | 0.008 | nd | |
| $R_1$ = 4-($C_6H_{13}O$)Ph, $R_2$ = H (*E*:*Z* = 20:80) | 412 | 391 | 0.004 | 442 | 0.044 |
| $R_1$ = 4-($C_6H_{13}O$)Ph, $R_2$ = $CH_3$ | nd | nd | | nd | |
| $R_1$ = *n*-hexyl, $R_2$ = H (*E*:*Z* = 40:60) | 416 | 389 | 0.018 | 443 | 0.071 |
| $R_1$ = *n*-pentyl, $R_2$ = $CH_3$ | nd | nd | | nd | |
| $R_1$ = *n*-butyl, $R_2$ = $C_2H_5$ | 389 | 423 | 0.002 | nd | |
| $R_1$ = $CH_2$Ph, $R_2$ = H (*E*:*Z* = 25:75) | 415 | 390 | 0.011 | 442 | 0.050 |

[a] All difference spectra were obtained with PB-treated rat liver microsomal suspensions (1 μM P450; 2 nmol P450 per mg protein) after addition of 100 μM oxime (mixture of *Z* and *E* isomers indicated above for aldoximes; for ketoximes, mixture of isomers obtained by reaction of the corresponding ketone with $NH_2OH$) (from dimethyl sulfoxide solutions) to the sample cuvette. Under these conditions, difference spectra of P450 Fe(III) appeared immediately and remained constant. Difference spectra corresponding to P450 Fe(II) complexes rapidly appeared after addition of 10 mM sodium dithionite to both cuvettes, reached their greatest intensity within 1 min, and then decreased with time. The indicated $\Delta A$ (442–490 nm) values correspond to the greatest intensity of the difference spectra and are expressed per 1 μM P450 per cm. [b] 20 μM (*Z*)- or (*E*)-benzaldoxime. [c] nd: not detected.

Table 4: Dehydration of Various Aldoximes by Rat Liver Microsomes in the Presence of Dithionite[a]

| aldoxime | initial rate of nitrile formation nmol $min^{-1}$ (nmol P450)$^{-1}$ |
|---|---|
| benzaldoxime (*E*:*Z* = 0:100) | 1.0 ± 0.4 |
| 4-(hexyloxy)benzaldoxime (*E*:*Z* = 20:80) | 0.8 ± 0.4 |
| heptanaldoxime (*E*:*Z* = 40:60) | 5.8 ± 2 |
| phenylacetaldoxime (*E*:*Z* = 25:75) | 12.5 ± 4 |

[a] Conditions as in Table 1 except that 200 μM aldoxime, 1 mM P450 (2 nmol P450 per mg of protein), and 10 mM dithionite (instead of NADPH) were used. Initial rates were measured by following the reactions at 37 °C during 10 min. Mean values ± SD from at least three experiments.

Table 5: Dehydration of (*Z*)- and (*E*)-Benzaldoxime by Microsomes from Yeast Expressing Human Liver P450 3A4 in the Presence of Dithionite[a]

| conditions | nitrile (μM) | % of CS + **2Z** |
|---|---|---|
| CS + **2Z** | 8.2 | 100 |
| CS + **2E** | <0.2 | <3 |
| CS + **2Z** – cyt $b_5$ | 5.4 | 66 |
| CS + **2Z** + CO | 0.0 | 0 |
| CS + **2Z** + miconazole (100 μM) | 5.4 | 66 |
| CS + **2Z** + dihydroergotamine (100 μM) | 0.0 | 0 |
| CS + **2Z** – microsomes | 0.0 | 0 |

[a] Anaerobic microsomes from the W(R) yeast strain expressing P450 NF25 (Peyronneau et al., 1992) (1 μM P450) in 0.1 M phosphate buffer pH 7.4 containing 10 mM sodium dithionite and 1 μM rabbit liver cytochrome $b_5$ were used (complete system = CS). Incubations with 100 μM **2Z** or **2E** were performed 30 min at 28 °C. Results are means ±15% from at least 3 experiments.

expressed in yeast (Peyronneau et al., 1992, 1993). Dehydration of **2Z** did not occur in the absence of dithionite or when using control yeast microsomes not expressing P450 NF25 cDNA in the presence of dithionite. Specific inhibitors of P450 NF25 like troleandomycin, dihydroergotamine, and bromocriptine (Peyronneau et al., 1993) greatly inhibited or completely suppressed this reaction (Table 5). Carbon monoxide also completely inhibited dehydration of **2Z**.

These results clearly show that a major P450 from human liver catalyzes the dehydration of a (*Z*)-aldoxime. The characteristics of this reaction, i.e., the only occurrence on the *Z* isomer, the requirement of a reducing agent to reduce P450 Fe(III), and the strong inhibition by P450 Fe(II) ligands like CO, as well as by P450 inhibitors like troleandomycin and dihydroergotamine, are very similar to those found for dehydration of **1** and **2Z** by rat liver microsomes.

## DISCUSSION

The cytochrome P450-dependent dehydration of aldoximes first observed in the case of butyraldoxime (De Master et al., 1992) appears as a rather general reaction as it was shown to occur in the case of several arylaldoximes (benzaldoxime and 4-(hexyloxy)benzaldoxime, Tables 1 and 2) and alkylaldoximes (heptanaldoxime and phenylacetaldoxime, Table 4). All these reactions are catalyzed by cytochrome P450 in its ferrous state as shown by the absolute requirement of NADPH under anaerobic conditions or of dithionite and the strong inhibitory effects of $O_2$ and CO (Tables 1, 2, and 5). It is noteworthy that these new P450 reactions involve P450 Fe(II) as a key active species and not P450 Fe(II)–$O_2$ or any subsequent intermediate of the usual catalytic cycle of P450-dependent monooxygenations (Ortiz de Montellano, 1986). Moreover, we have shown that P450 3A4, a major P450 isozyme in human liver, is able to catalyze this reaction with characteristics similar to those of rat liver microsomal P450s (Table 5).

This P450-dependent dehydration was found to only occur on (*Z*)-aldoximes, as demonstrated in the case of benzaldoxime and 4-(hexyloxy)benzaldoxime. (*Z*)-Aldoximes strongly interact with P450 Fe(II) with formation of 442-nm absorbing complexes (Table 3). During dehydration of (*Z*)-aldoximes, consumption of the aldoxime, disappearance of the P450 complex, and formation of the corresponding nitrile are concomitant (Figure 3). On the contrary, (*E*)-aldoximes are not dehydrated under identical conditions and do not form 442-nm absorbing complexes. It is thus tempting to propose that these complexes are intermediates in the dehydration of aldoximes.

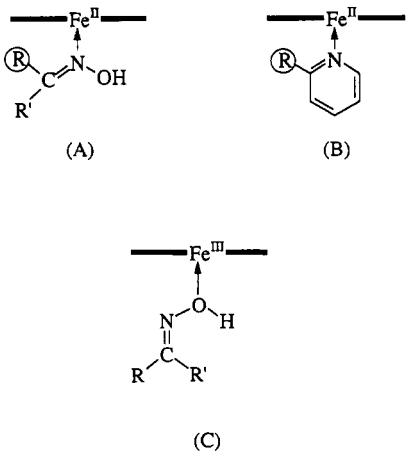


FIGURE 4: Possible structures for the complexes formed between (*Z*)- and (*E*)-aldoximes with P450 Fe(III) (C) and between (*Z*)-aldoximes and P450 Fe(II) (A). The P450 Fe(II)–(*Z*)-aldoxime complexes (A) ($\lambda_m$ = 442 nm) (R = H) are compared to P450 Fe(II)–pyridine complexes (B). When R ≠ H ((*E*)-aldoximes, ketoximes, or ortho-substituted pyridines) complex formation is prevented because of a steric interaction between the R group and the heme.

*Nature of the 442-nm Absorbing P450–Aldoxime Complexes.* As far as the nature of these complexes is concerned, the position of their Soret peak is similar to that of complexes between P450 Fe(II) and nitrogen-containing ligands like amines, pyridines and nitrogen-containing heterocycles (Dawson & Sono, 1987). They are not complexes formed between P450 Fe(II) and amines $RCH_2NH_2$, which could have been formed upon reduction of aldoximes RCH=NOH in the medium, as (i) benzylamine fails to give any 442-nm absorbing complex under conditions where (*Z*)-benzaldoxime gives an intense 442-nm spectrum, (ii) 4-(hexyloxy)benzaldoxime also gives an intense 442-nm spectrum whereas 4-(hexyloxy)-benzylamine leads to a very different ($\lambda_{max}$ = 447 and 428 nm) and much weaker spectrum, and (iii) in a general manner, the aldoxime-derived complexes immediately appear after addition of aldoximes to dithionite-reduced microsomes contrary to what could be expected if they would derive from a reduction of aldoximes before coordination of the corresponding amines to P450 Fe(II). The 442-nm absorbing complexes are not derived from the binding to P450 Fe(II) of hydroxylamines $RCH_2NHOH$ which could have been formed upon reduction of aldoximes RCH=NOH in the reducing medium, as interaction between *N*-alkylhydroxylamines $RCH_2NHOH$ with P450 Fe(II) has been previously studied and did not lead to complexes absorbing around 442 nm (Mansuy et al., 1978). Thus, it is very likely that the observed 442-nm absorbing complexes are formed by simple binding of the nitrogen atom of aldoximes to P450 Fe(II). Such a structure explains why only the oximes bearing a hydrogen substituent in position trans to the OH group, namely the (*Z*)-aldoximes, can give 442-nm absorbing complexes. (*E*)-Aldoximes and all ketoximes have an alkyl or aryl substituent in that position which prevents their approach to the porphyrin ring because of steric hindrance (Figure 4); therefore, they do not give 442-nm absorbing complexes (Table 3). These complexes are comparable to the ones formed between P450 Fe(II) and nitrogen-containing heterocycles like pyridines or imidazoles (Figure 4). Not only do they exhibit similar positions of their Soret peak but also their formation is prevented in case of the presence of a substituent different from H in ortho position of the nitrogen atom. Binding of the oxygen atom of oximes to P450 Fe(II) would not lead to such severe steric restrictions, and one would expect that

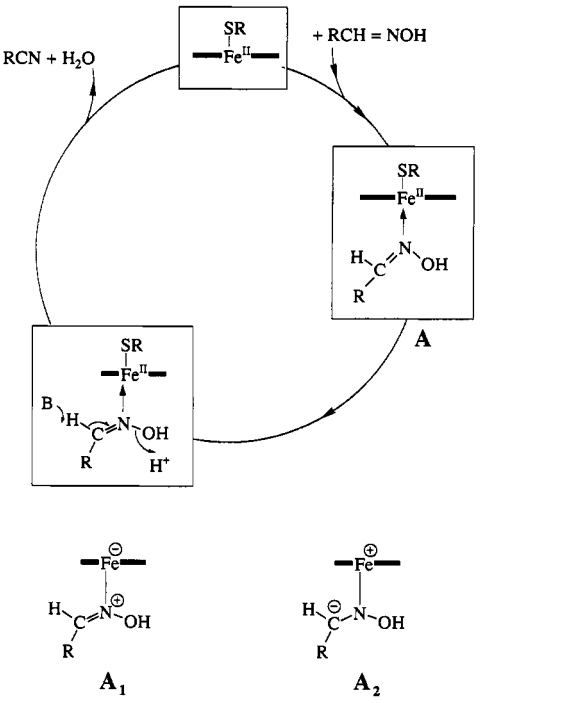


FIGURE 5: Possible catalytic cycle for the P450-dependent dehydration of (*Z*)-aldoximes. B is a basic amino acid residue of P450 active site. RS is the cysteinate P450 ligand. $A_1$ and $A_2$ are possible mesomeric forms of the 442-nm absorbing complex A. They are starting points for two possible mechanisms indicated in the text for the catalysis of aldoxime dehydration in complex A.

(*E*)-aldoximes could also give such O-bonded P450 complexes. Anyway, oxygen-containing ligands like alcohols or ethers are not known to bind to P450 Fe(II) whereas they bind to P450 Fe(III) with the appearance of reverse type I spectra characterized by a peak around 415 nm and a trough around 390 nm (Dawson and Sono, 1987). Accordingly, both (*E*)- and (*Z*)-aldoximes interact with microsomal P450 Fe(III) with the appearance of reverse type I difference spectra (Table 3, Figure 4).

*Possible Mechanism for P450-Dependent Dehydration of (Z)-Aldoximes (Figure 5).* The aforementioned results show that (*Z*)- and (*E*)-aldoximes bind to P450 Fe(III) by their oxygen atom. After reduction of P450 Fe(III), only (*Z*)-aldoximes can bind to P450 Fe(II) by their nitrogen atom with formation of 442-nm absorbing complexes. This binding to P450 Fe(II) appears to be essential for dehydration of aldoximes, as (*E*)-aldoximes that do not give such P450 complexes are not dehydrated. Two mechanisms may be proposed to explain the key role of P450Fe(II) for the catalysis of aldoxime dehydration in these complexes. In the first one, the coordination of the oxime nitrogen to P450 Fe(II), which could lead to a charge transfer from the C=N moiety to the iron (illustrated in mesomeric form $A_1$ of complex A in Figure 5), would make the CHR hydrogen more acidic and facilitate its abstraction by a basic amino acid residue of P450 active site. This proton abstraction should be followed by a loss of the oxime OH group assisted by its protonation. The second possible mechanism is based, on the contrary, on a possible charge transfer from the iron to the aldoxime N=C bond. This is likely because of the high electron richness of P450 Fe(II) due both to its ferrous state and to the presence of its endogenous axial cysteinate ligand. Such a charge transfer should lead to the appearance of a negative charge on the aldoxime carbon atom (mesomeric form $A_2$ in Figure 5) and subsequent elimination of the aldoxime OH group, presumably after protonation. The resulting complex, which may be

written at least formally as a P450 Fe(IV)—N=CHR complex, contains a highly activated hydrogen atom $\beta$ to the electrophilic iron(IV) species and should lose this hydrogen atom as a proton to give RCN and regenerate P450 Fe(II). Additional data are necessary to decide between these two mechanisms. However, the faster dehydration observed with alkylaldoximes than with arylaldoximes (Table 4), which could be explained by a faster cleavage of the aldoxime N–O bond of the former substrates because of the greater instability of their carbanionic intermediate ($A_2$ in Figure 5), would be in favor of the second mechanism. On the contrary, in the first mechanism, one would have expected an easier abstraction of the aldoxime CHR hydrogen of arylaldoximes (benzylic hydrogen) than of alkylaldoximes.

A possible physiological role of this P450-dependent dehydration of aldoximes remains to be discovered. However, it is noteworthy that (*p*-hydroxyphenyl)acetaldoxime is an intermediate in the biosynthesis of cyanogenic glucosides from tyrosine in higher plants (Halkier et al., 1989a). The involvement of at least one cytochrome P450 in that reaction has been proposed (Halkier et al., 1989b). Interestingly, one of the last steps of this biosynthetic pathway is the dehydration of (*Z*)-(*p*-hydroxyphenyl)acetaldoxime into the corresponding nitrile. This step is catalyzed by microsomes of sorghum seedlings in the presence of NADPH (Halkier et al., 1989a). Further studies are necessary to clarify this reaction in plants and to know the importance of P450-catalyzed dehydration of aldoximes in living organisms.

## ACKNOWLEDGMENT

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