A Beckmann-Type Dehydration of *n*-Butyraldoxime Catalyzed by Cytochrome P-450

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Summary: *n*-Butyraldoxime undergoes a unique NADPH-dependent, Beckmann-type dehydration catalyzed by rat liver microsomes to give *n*-butyronitrile.

n-Butyraldoxime (1) is known to inhibit hepatic aldehyde dehydrogenase (AlDH, EC 1.2.1.3) in vivo but not in in vitro, suggesting that metabolism is required for enzyme inhibition.[1] We have shown that the enzyme system responsible for the conversion of 1 to an inhibitor of AlDH is the hepatic microsomal mixed function oxidase[2] and that catalase, the enzyme that bioactivates cyanamide,[3] another potent in vivo AlDH inhibitor, is not involved.

In our quest to identify the reactive species from 1 that inhibits AlDH, we relied on GC and GC–MS to identify the volatile metabolic end products formed in the oxidation of 1 catalyzed by phenobarbital-induced rat liver microsomes in vitro. Two major GC peaks representing metabolites derived from 1 with retention times of 1.8 and 5.3 min were observed on a Tenax GC column. The latter was identified as the N-oxidation product of 1, viz. nitrobutane (4), by identity of GC retention times and GC–MS data with an authentic sample of 4. The second metabolite was identified unequivocally by GC retention time data and by GC–MS [70 eV: *m/z* 41 ($C_3H_5^+$, base peak); 29 ($C_2H_5^+$); 27 $(C_2H_3)^+$] as *n*-butyronitrile (3).[4] The enzymatic formation of 3, a Beckmann-type dehydration product of 1,[5] appears to be novel and unprecedented. These reactions are catalyzed by cytochrome P-450 because (a) NADPH was required for the formation of 3 and 4, (b) product formation was not observed with heat-denatured rat liver microsomes, (c) CO inhibited both reactions, and (d) 1-benzylimidazole, an inhibitor of cytochrome P-450,[6] also blocked the formation of 3 and 4 (Table I). Preincubation of the microsomes in the absence of NADPH did not affect the formation of 3 or 4 (data not shown), suggesting that the flavin monooxygenase system was not involved.[7]

Nitrobutane (4) is formed by the direct oxygenation of the oximino nitrogen (Scheme I) and was the expected nitrogen oxidation product of 1. 1-Phenyl-2-propane oxime (isomeric to the corresponding C-nitroso compound), a ketoxime and a metabolite of amphetamine[8] which itself is derived from the intermediate *N*-hydroxyamphetamine,[9] has also been shown to be oxidized to a nitro compound, viz. to 2-nitro-1-phenylpropane,[10] by rat liver microsomes.

Evidence providing mechanistic insights for this dehydration reaction of *n*-butyraldoxime (1) catalyzed by cytochrome P-450 include the following: (a) molecular oxygen inhibited nitrile 3 but not nitrobutane (4) formation, (b) only a catalytic amount of NADPH was required for the generation of 3 from 1 under anaerobic conditions, and (c) NADPH could be replaced by sodium dithionite (Table II). Under anaerobic conditions, continuous generation of NADPH was not required, since 100 nmol of NADPH without added glucose 6-phosphate and glucose 6-phosphate dehydrogenase (EC 1.1.1.49) supported the reaction (Table II). This suggests that once NADPH reduces cytochrome P-450 to the ferrous state, the reaction proceeds without further action by NADPH. The participation of cytochrome P-450 in its ferrous state (Fe^{2+}) was further supported by the demonstration that dithionite-reduced

Table I. The Conversion of *n*-Butyraldoxime (1) to Butyronitrile (3) and Nitrobutane (4) Catalyzed by Phenobarbital-Induced Rat Liver Microsomes under Aerobic Conditions

expl condns[a]	butyronitrile (3) formed (% of control)	nitrobutane (4) formed (% of control)
complete system (CS)	100 ± 1	100 ± 4
CS minus $NADP^+$[b]	<1	<1
CS minus microsomes	<1	<1
CS with heat-treated microsomes[c]	<1	<1
CS plus CO (5 mL)	<1	45 ● 1
CS plus 1-benzylimidazole (1.0 mM)[d]	12 ± 1	10 ● 5

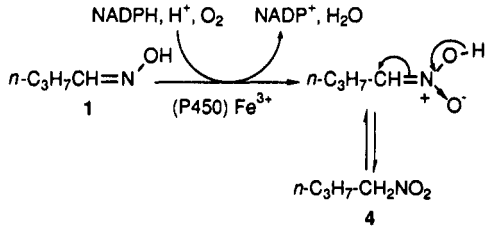
[a] Reaction mix containing 10 mM 1, 5.0 mM $MgCl_2$, 16 mM KCl, 100 mM potassium phosphate (pH 7.4), and 2.5 mM glucose 6-phosphate (G-6-P) was preincubated for 5 min at 37 °C followed by the addition of 1.0 μmol of $NADP^+$, 5.0 U G-6-P dehydrogenase, and 300 μL of phenobarbital-induced rat liver microsomes (reconstituted to 1.0 mL per g wet weight tissue in 100 mM potassium phosphate buffer, pH 7.4) in a total volume of 1.0 mL in a 22 mL glass GC septum vial. After a 5-min incubation period, the reaction was stopped by the addition of 0.1 mL of 5.5 N $HClO_4$, and the samples were stored on ice until analyzed by headspace GC. [b] Converted to NADPH by the NADPH generating system. [c] Microsomes were heated for 15 min at 100 °C. [d] Concentration of 1 was 2.0 mM.

Table II. *n*-Butyronitrile (3) Formation under Conditions that Favor the Ferrous State of Cytochrome P-450

expl condns[a]	butyronitrile (3) formed (nmol/5.0 min)	nitrobutane (4) formed (nmol/5.0 min)
NADPH, N_2 atmosphere	2040 ● 36	<1
NADPH, air atmosphere	456 ● 26	35.0 ± 0.9
NADPH, O_2 atmosphere	13 ± 2	36.5 ± 0.9
N_2 atmosphere[b]	<1	<1
100 nmol NADPH,[b] N_2 atmosphere	1103 ● 67	<1
10 mM $Na_2S_2O_4$,[b] N_2 atmosphere	1870 ± 12	<1

[a] Reaction conditions were as described for the complete system in Table I with the exceptions noted. [b] $NADP^+$, G-6-P, and G-6-P dehydrogenase omitted.

Scheme I



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Scheme II

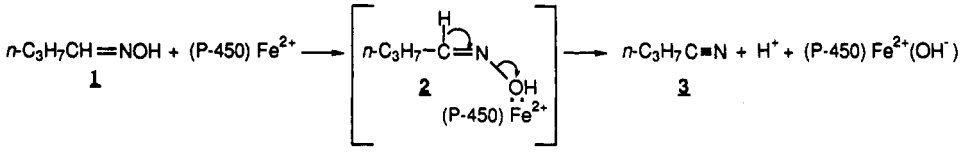

Table III. Conversion of *n*-Butyraldoxime (1) to Butyronitrile (3) Catalyzed by Sodium Dithionite (DT) Reduced Hematin under Anaerobic Conditions

Fe source (1.0 mM)	pH	N	butyronitrile (3) formed[a] (nmol/15 min)
hematin	6.0	6	<1
hematin	7.4	6	<1
hematin + DT	6.0	5	105 ± 5
hematin + DT	7.4	6	222 ± 8
$Fe_2(SO_4)_3$	6.0 (or 7.4)	3 (3)	<1
$FeSO_4$	6.0 (or 7.4)	3 (3)	<1

[a] Reaction mix containing 100 mM potassium phosphate buffer and an Fe source (as indicated) was preincubated for 5 min at 37 °C followed by the addition of sodium dithionite (DT; 10 μmol, where indicated) and 10 μmol of 1 in a total volume of 1.0 mL. After a 15-min incubation period under N_2, the reaction was stopped by the addition of 0.1 mL of 5.5 N $HClO_4$ and the samples were stored on ice until assayed by head space GC.

hematin (Fe^{2+}) also catalyzed this reaction, albeit at a rate of 0.04% of the NADPH-supported cytochrome P-450 reaction, based on nmol Fe. Hematin itself (Fe^{3+}), $Fe_2(SO_4)_3$, or $FeSO_4$ was ineffective in catalyzing this reaction (Table III). The difference spectra for 1 with Fe^{2+} cytochrome P-450 showed a trough at 411 nm, a characteristic of ligand binding to reduced cytochrome P-450,[11] and a peak at 444 nm demonstrating that substrate binding occurs with Fe^{2+} cytochrome P-450. The substrate binding spectra for 1 with Fe^{3+} cytochrome P-450 was reverse Type I.[12]

We envision this cytochrome P-450 catalyzed Beckmann-type dehydration to proceed via a mechanism involving the interaction of the oximino oxygen atom with ferrous iron of cytochrome P-450, as shown in Scheme II. The coordinated complex (hypothetical intermediate 2) provides the driving force for the elimination of H_2O from the oxime 1.

Although aldoximes are known to dehydrate to the corresponding nitriles under Beckmann conditions,[5] we are unaware of any aldoximes being converted to nitriles by cytochrome P-450. In the present case, 1 was converted to 3 with the latter nitrile product retaining the carbon skeleton of the original oxime. Highly branched ketoximes give abnormal Beckmann products, *including nitriles*, accompanied by fission of the carbon skeleton.[5] We present our preliminary observations at this time to encourage further studies (by others) with additional oxime substrates using purified isoforms of cytochrome P-450 and/or model cytochrome P-450 mimics.

Neither *n*-butyronitrile (3) nor nitrobutane (4) inhibited yeast AlDH directly or when coupled with the cytochrome P-450/NADPH system. These results suggest that another oxidative bioactivation pathway must exist for the generation of the active AlDH inhibitor from 1. We are presently attempting to identify this active species.

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Supplementary Material Available: GC–MS spectral data for authentic and enzyme-generated 3 (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(4) The GC–MS spectra of 3 are available as supplementary material.
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Metal-Catalyzed Decarbonylation of Primary Aldehydes at Room Temperature

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Summary: The metal-catalyzed decarbonylation of primary aldehydes has been achieved at room temperature in THF solvent by utilization of catalytic amounts of $Rh(PPh_3)_3Cl$ (2, 5 mol %), in conjunction with stoichiometric amounts of diphenylphosphoryl azide (DPPA, 4).

The development of a mild, efficient procedure for the decarbonylation of aldehydes would represent an impor-

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