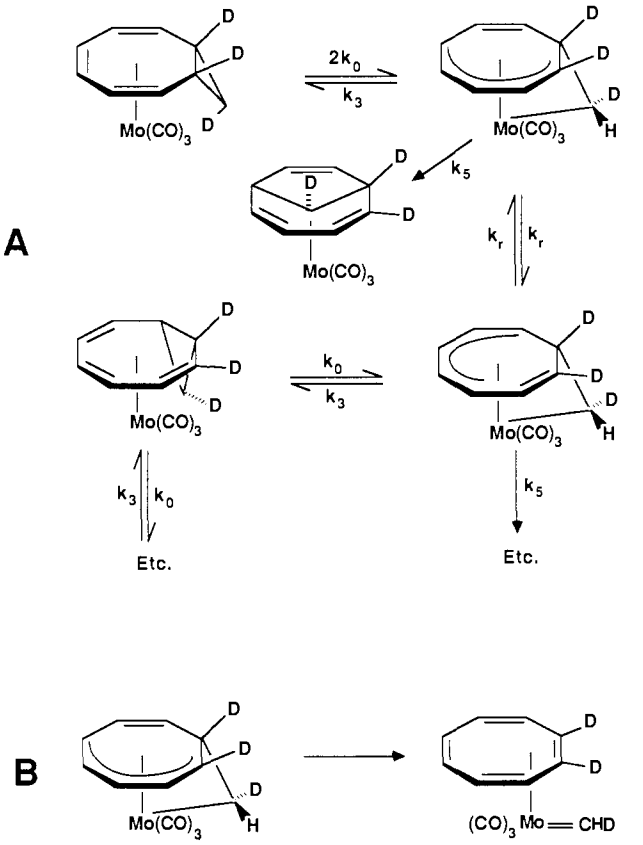

Figure 4. Mechanisms considered for label migration around the eight-membered ring. These mechanisms are rigorously excluded for the degenerate rearrangement and disfavored, but not rigorously excluded, for product formation.

The bicyclo[4.2.1]nonatriene is formed with retention of configuration at C9, and so it *could* arise from direct involvement of the metal in C–C cleavage. However, since the same bond must be broken for the degenerate [1,7] shift and for this process, it seems more economical to suppose that neither process involves oxidative addition to the metal. This view is further supported by the observation that Cr, Mo, and W complexes rearrange with rate constants that differ by a factor of only 12.6 at 111.1 °C ($k_{Cr}/k_{Mo} = 5.80$, $k_{Mo}/k_{W} = 2.18$). One might have expected a greater range of rates for oxidative addition, especially between the first- and second-row metals. Furthermore, the [1,5] shift that one would have to invoke for the reaction without direct oxidative addition is again known for substituted bicyclo-[6.1.0]nonatrienes, although it exhibits a slight preference for inversion of configuration in the one case where the stereochemistry has been studied.[10]

In summary, the degenerate [1,7] shift observed in (bicyclo-[6.1.0]nonatriene)tricarbonylmolybdenum is a process that definitely does not involve the metal in C–C cleavage. The most economical explanation of the overall rearrangement process is that complexation to the metal suppresses the epimerization and conversion to dihydroindene observed for the free hydrocarbon and thereby allows access to the higher energy [1,7]- and [1,5]-sigmatropic migrations, which occur without direct assistance from the metal.

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Functionalized Nitrogen Atom Transfer Catalyzed by Cytochrome P-450[1]

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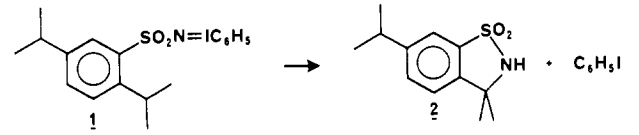
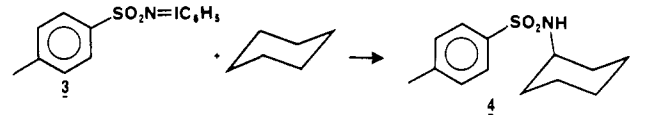
The cytochromes P-450 are heme iron-containing enzymes that catalyze NADH- and O_2-dependent substrate hydroxylations, olefin epoxidations, heteroatom dealkylations, and oxidations,[3] as well as NADH-dependent reductive dehalogenations.[4] The activation of molecular oxygen via an iron–oxene intermediate with subsequent oxygen transfer to substrate has been implicated in the P-450 reaction mechanism.[3] P-450 is also able to utilize oxygen atom donors such as iodosobenzene[5] and tertiary amine oxides[6] for substrate oxygenation. Groves and others have reproduced this latter process with metalloporphyrin model systems.[7] Recently,[8,9] metalloporphyrins have been shown to catalyze the transfer of a functionalized nitrogen atom from a tosylimide analogue of iodosobenzene (**1** → **2**; **3** → **4**). The latter reaction





has been attempted using cytochrome P-450-LM2 as the catalyst, but cyclohexanol was found as the only product.[10] We wish to report the successful cytochrome P-450 catalyzed transfer and incorporation of a functionalized nitrogen atom into a C–H bond. This enzymatic activity, previously unobserved with P-450 or any other hemoprotein, has also been found to be isozyme-dependent.

Using purified rabbit liver microsomal cytochrome P-450-LM3,4[11,12] as the catalyst, we have studied the intramolecular transfer of nitrogen in [[(2,5-diisopropylphenyl)sulfonyl]imi-

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(12) The LM3,4 isozyme preparations (referred to as LM4 by Coon et al.[11]) typically had specific contents of 10–13 nmol of P-450 (mg prot)$^{-1}$ and had isozyme ratios of LM3:LM4 = 2:5 as determined by denaturing polyacrylamide gel electrophoresis. The LM2 isozyme preparations typically had specific contents of 10 nmol of P-450 (mg prot)$^{-1}$ with 5–10% P-420 contamination.

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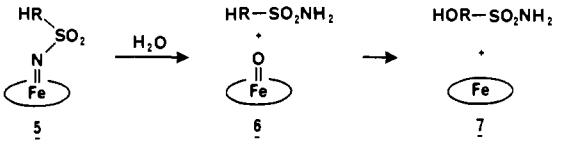
no]phenyliodane (**1**) to yield 2,3-dihydro-3,3-dimethyl-6-isopropyl-1,2-benzisothiazole 1,1-dioxide (**2**) as the amidation product. This reaction has been previously reported with metalloporphyrins and other transition-metal complexes as catalysts.[8b] As with the model systems, we find that intramolecular nitrogen transfer (**1** → **2**) proceeds more rapidly and yields greater amounts of amidation product than the analogous intermolecular reaction (**3** → **4**).

Cytochrome P-450-LM3,4 has been found to catalyze intramolecular amidation (**1** → **2**), as evidenced by multiple turnovers (2.2) of the enzyme under optimal conditions.[13] The reaction rate, as determined by product **2** formation,[13b] is linearly dependent on P-450 concentration (0–1 μM), time (0–3 min), and substrate (**1**) concentration (0–0.53 mM), with a turnover number[14] of 1.0 for the LM3,4 isozyme mixture, a value that is a lower limit since saturation kinetics were not observed even at the limit of substrate solubility (ca. 0.7 mM). Enzyme samples were extensively dialyzed to remove free metal ions, which are known to catalyze the reaction under study,[8b] and the low background activity of the final dialysis buffer was measured in order to determine net product formation.

The integrity of P-450 is crucial to its catalytic activity in the amidation reaction. A 29%/71% mixture of P-450/P-420-LM3,4[15] has an amidation activity equal to 16 ± 7% that of untreated P-450. Within error, all of this activity can be accounted for by the P-450 present. Extensively denatured[16] P-450-LM3,4 shows no net amidation activity.

The amidation activity of P-450 has also been found to be isozyme-dependent. The turnover number for intramolecular amidation with the LM2 isozyme[12] is 3% that of the LM3,4 mixture and likely results from trace (2–5%) amounts of the LM3,4 isozymes present in the LM2 sample. We have also examined the intermolecular amidation reaction (**3** → **4**) that White and McCarthy[10] attempted to study using P-450-LM2. We have found that cyclohexanol is exclusively produced with the LM2 catalyst, confirming the earlier results.[10] However, we have observed amidation product in addition to, but in smaller quantities than, cyclohexanol with either microsomes or the LM3,4 isozyme mixture (turnover numbers of 0.16 and 0.31, respectively, for the formation of **4**). For comparison, White and McCarthy have reported a turnover number of 4.0 for the P-450-LM2-catalyzed conversion of **3** to cyclohexanol. Similar isozyme dependence on the other P-450 activities has been well characterized.[17]

A second substance has also been detected in the intramolecular amidation reaction mixture. Since its relative amount[18] has been found to increase in parallel to **2**, the unknown component appears to be a reaction product. The chemical-ionization mass spectrum of this compound gave ion peaks at *m/e* 258 (M + 1) and 240 (M + 1 − H_2O), while its electron-impact mass spectrum showed fragment ions of similar masses as those of the free sulfonamide, 2,5-diisopropylbenzenesulfonamide (242 g/mol). These characteristics are consistent with a monooxygenated form of the free sulfonamide, likely produced from the hydrolysis of the iron–nitrene intermediate (**5** → **6**),[19] followed by hydroxylation of the resulting amide (**6** → **7**). The presence of a hydroxylated product



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is also consistent with the results of the intermolecular amidation reaction studied by White and McCarthy.[10]

In summary, liver microsomal cytochrome P-450-LM3,4 has been found to catalyze intra- and intermolecular functionalized nitrogen atom transfer. Such a catalytic activity has not been previously demonstrated with P-450. Earlier attempts to examine this type of reaction with P-450-LM2 as the catalyst were unsuccessful, although a hydroxylation activity was observed.[10] The intramolecular amidation reaction has been found to be P-450 isozyme dependent and to proceed only in the presence of the intact and active form of the enzyme.

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(13) (a) Reaction mixtures typically containing 0–12 nmol of P-450, 40 μmol of potassium phosphate, and 0.02–0.10 mL of glycerol in a total volume of 2.0 mL at pH 7.4 and a temperature of 30 °C. The reaction was initiated by adding 0.1 mL of a 10 mM solution of **1** in MeOH and terminated by addition of excess $NaHSO_3$. The organic products were extracted with CH_2Cl_2, which was concentrated and analyzed on a Finnigan 4021 GC/MS. (b) Product identification was achieved by comparing the mass spectra of the eluting peaks with that of pure **2**. Quantitation of **2** was based on the *m/e* 224 ion peak area, relative to the *m/e* 171 ion peak area of *p*-toluenesulfonamide, the internal standard.

(14) Turnover number is the reaction rate in units of nmol of product/nmol of P-450/min.

(15) P-420 is the enzymatically inactive form of P-450[3a] and was prepared by KSCN (1 M) treatment followed by extensive dialysis in the absence of KSCN.

(16) Heated at 60 °C for 15 min.

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(18) Quantitation was not possible in the absence of a pure compound.

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Additions and Corrections

Synthesis and Characterization of a New Fe/Mo/S Cluster Containing the $[Fe_6Mo_2S_6]^{3+}$ Core. A Precursor to a Possible Structural Analogue for the Fe/Mo Site in Nitrogenase [*J. Am. Chem. Soc.* **1985**, *107*, 5005–5006]. D. COUCOUVANIS* and M. G. KANATZIDIS

Page 5006: The caption of Figure 1 should read as follows: Proposed structure for the $[Fe_6S_6(OPh\text{-}p\text{-}CH_3)_6(Mo(CO)_3)_2]^{3-}$ trianion.[16]