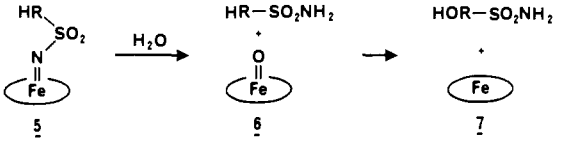
no]phenyliodane (**1**) to yield 2,3-dihydro-3,3-dimethyl-6-isopropyl-1,2-benzisothiazole 1,1-dioxide (**2**) as the amidation product. This reaction has been previously reported with metalloporphyrins and other transition-metal complexes as catalysts.[8b] As with the model systems, we find that intramolecular 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.

(17) (a) Guengerich, F. P.; Dannan, G. A.; Wright, S. T.; Martin, M. V.; Kaminsky, L. S. *Biochemistry* **1982**, *21*, 6019–6030. (b) Coon, M. J.; Black, S. D.; Koop, D. R.; Morgan, E. T.; Tarr, G. E. "Microsomes, Drug Oxidations, and Drug Toxicity"; Sato, R., Kato, R., Eds.; Wiley-Interscience: New York, 1982; pp 13–23. (c) Kitagawa, H.; Omori, S.; Kitada, M.; Kanakubo, Y. *Ibid.*; pp 97–98.

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

(19) Hydrolysis of the presumed iron–oxene intermediate has been implicated in P-450-catalyzed hydroxylations using iodosobenzene: Macdonald, T. L.; Burka, L. T.; Wright, S. T.; Guengerich, F. P. *Biochem. Biophys., Res. Commun.* **1982**, *104*, 620–625.

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]