cluster in the enzyme aconitase.[35] The isomer shift of the oxidized Fe protein cluster is 0.45 mm/s, typical of $[4Fe\text{-}4S]^{2+}$ clusters with a four-coordinate environment about each iron.

Thus, the Mössbauer results restrict the possible binding arrangements of the cluster to those with four-coordinate iron sites. The EXAFS results further restricts the possibilities to clusters with either four external sulfur ligands or to those with a single oxygen-for-sulfur substitution. Finally, since the Fe protein consists of two identical subunits and it appears that the cluster is bound between them, it is unlikely that unsymmetrical binding arrangements, such as those entailed by a single oxygen-for-sulfur substitution, would occur.

The ability to observe differences in Fe–S distances in these data indicate that similar or greater differences, arising from other phenomena, would be observed if they were actually present. It appears, however, that the $S = {}^1/_2$, $S = {}^3/_2$ spin-state conversion requires a change in the average Fe–S bond length of less than ~0.03 Å. The data of both $S = {}^1/_2$ (Av2/HMPA) and $S = {}^3/_2$ (Av2/urea) states fit to Fe–S and Fe–Fe terms with similar distances and coordination numbers. This indicates that the clusters in both spin states are of the same type, with no major cluster rearrangement and no major change in the average Fe–S bond length. In addition, there is no indication that additional ligands are coordinating to the cluster in order to effect spin-state conversion. Of course, small changes in the coordination environment, such as an oxygen ligand binding to one of the iron atoms of the cluster, may have gone undetected. However, invoking minor coordination changes in the cluster to effect these spin-state changes seem unnecessary at present, given the evidence (see Introduction) that similar changes in the magnetic properties of other $[4Fe\text{-}4S]^+$ clusters are caused by only minor changes in cluster structure. We suspect that minor changes in bond lengths, but possibly more substantial changes in bond angles, are required for the Fe protein spin-state changes. Similar conclusions can be drawn for the effect of the MgATP-induced conformational change on the cluster structure, since there were no noticeable changes in the EXAFS data of the MgATP-bound protein; the cluster structure probably changes very little.

The Fe–Fe terms of the Fe protein data have significantly larger DW factors (average of 0.10 Å) than the model compound terms (average of 0.08 Å). Such differences diminish the ability of the FABM method to accurately estimate coordination numbers.[29] In the present case, the average calculated Fe–Fe coordination number of 2.4 for the Fe protein data is somewhat lower than the expected number, 3.0. The differences in the DW factors appear to have *lowered* the calculated coordination number of the protein; those spectra with DW factors closer to those of the model compound data had coordination numbers closer to 3. We had thought that clusters in protein may have higher DW factors generally. However, we found that the DW factor in the ferredoxin from *C. pasteurianum*, which contains two [4Fe–4S] clusters, was quite close to the model compound value, and yielded an Fe–Fe coordination number of 3.3.[36] We cannot, at present, determine whether the large DW factors in the Fe protein data result from a distortion in the cluster or are artifacts, since this parameter is difficult to measure accurately.

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Registry No. $(Et_4N)_2[Fe_4S_4(SPh)_4]$, 55663-41-7; $(Et_4N)_3[Fe_4S_4(SPh)_4]$, 63115-82-2; nitrogenase, 9013-04-1.

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Methanolysis of ((Tosylimino)iodo)benzene

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The hypervalent iodine compound ((tosylimino)iodo)benzene (TosN=IPh) was found to solvolyze in methanol to *p*-toluenesulfonamide and (dimethoxyiodo)benzene, the components of the solution being identified by IR, HPLC, UV, and NMR methods. The solvolysis reaction is a dynamic equilibrium, easily reversible by addition of either product. NMR observation of signals representing TosN=IPh and tosylamine, respectively, at several concentrations of TosN=IPh allowed an estimation of the solvolysis equilibrium constant, $K_s = [TosNH_2][PhI(OMe)_2]/[TosN{=}IPh] = 0.70 \pm 0.10$ M. These results explain the previous observations of several workers that oxidation of substrates with iminoiodinanes catalyzed by metalloenzymes yields an *oxygen* insertion product (alcohol or epoxide) rather than a *nitrogen* insertion product (*N*-alkyl sulfonamide).

Investigations of various redox metalloenzymes have frequently found the use of artificial oxidants to have certain advantages over the use of the natural oxidants for these enzymes. Several hypervalent iodine compounds, including iodosylarenes,[1] (diacetoxyiodo)arenes,[1b] and iminoiodinanes,[2] have been used as artificial oxidants for cytochrome P-450 enzymes. Iodosylbenzene[3] and ((tosylimino)iodo)benzene[4] have also been used in studies of iron bleomycin. Iodosylbenzene[5] and ((tosylimino)iodo)benzene[6] have been favored as oxidants for iron porphyrin based hemeprotein model systems by many workers. Several studies have reported lability of the atom (either oxygen or nitrogen) nominally to be transferred to substrate via the metalloenzyme. For instance, complete incorporation of solvent water into the alcohol product was reported for the iodosylbenzene/P-450 oxidations.[1c,d,2a]

$$PhI{=}O + RH \xrightarrow[H_2{}^*O]{\text{P-450}} PhI + R^*OH \qquad (1)$$

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Similarly, partial or complete "exchange" of solvent water and nitrogen was reported in the P-450/iminoiodinane system[2] and the Fe bleomycin/iminoiodinane system.[4]

$$\text{TosN=IPh} + \text{RH} \xrightarrow[H_2{}^*O]{\text{P-450}} \text{PhI} + \text{TosNH}_2 + \text{R*OH} \quad (2)$$

A potential explanation for some of these results is found in the work of Schardt and Hill,[7] who reported that iodosylbenzene "dissolves" in methanol via solvolysis to form (dimethoxyiodo)-benzene.

$$\text{PhI=O} + 2\text{MeOH} \rightarrow \text{PhI(OMe)}_2 + \text{H}_2\text{O} \quad (3)$$

We now report on analogous solvolytic reaction of ((tosylimino)iodo)benzene.

Experimental Section

Chemicals. (((*p*-Tolylsulfonyl)imino)iodo)benzene and (((phenylsulfonyl)imino)iodo)benzene were prepared as described in detail by White and McCarthy.[2a] The infrared spectrum (KBr) of the phenylsulfonyl derivative was 3060 (w), 1579 (w), 1565 (m), 1469 (m), 1441 (s), 1270 (s), 1237 (s), 1115 (s), 1077 (s), 999 (m), 933 (m), 868 (s), 735 (s), 719 (m), 690 (m), 655 (m) cm^{-1}. Iodosylbenzene was prepared from (diacetoxyiodo)benzene.[8] Baker reagent grade methanol was used in all experiments except those in which deuteriated methanol was used (99.5% D from Aldrich Chemical Co.). Other chemicals were commercial products.

Analytical Procedures. Infrared spectra were recorded on a Perkin-Elmer 735 Spectrometer. NMR spectra were determined at 60 MHz on a Varian EM-360 instrument. High-pressure liquid chromatography was conducted on a Perkin-Elmer Series 4 liquid chromatograph using a Perkin-Elmer HS-5 C18 reverse-phase column (4.6 mm × 12.5 cm) and an Isco V4 absorbance detector. Optical spectra were recorded in 1-cm path length cuvettes with a Varian-Cary 219 spectrophotometer.

NMR Determination of the Equilibrium Constant. A stock solution of solid TosN=IPh (92 mg) in CD_3OD (1.85 mL) was prepared. *tert*-Butyl alcohol (3.5 mg) was added to an internal standard for quantitization. The final volume of the solution was 2.0 mL. Three dilutions in CD_3OD were prepared, giving four solutions of nominal TosN=IPh concentrations 0.13, 0.095, 0.063, and 0.045 M. After the NMR spectrum of each solution was recorded, the region from 0 to 3 ppm was repeatedly integrated. The absolute equilibrium concentration of Tos-$N-H_2$ was determined from the relative intensities of the methyl singlet of $TosNH_2$ and the methyl singlet of *tert*-butyl alcohol. The ratio of Tos-NH_2 to TosN=IPh was similarly determined from the intensities of the two methyl singlets near 2.5 ppm. The temperature of the NMR probe was 27 °C.

Results

Several similarities are found in the physical and chemical properties of PhI=O and TosN=IPh. Both are isolated as pale yellow, noncrystalline solids without distinctive melting points. They are essentially insoluble in nonnucleophilic organic solvents or in water but are readily dissolved by methanol. Each has a hypervalent iodine atom, making them moderately good oxidants. Given the polymeric nature of solid PhI=O,[7] we thought it likely that TosN=IPh was also a polymeric solid, solvolyzing to (dimethoxyiodo)benzene and *p*-toluenesulfonamide in the presence of methanol.

$$\text{TosN=IPh} + 2\text{MeOH} \rightleftharpoons \text{TosNH}_2 + \text{PhI(OMe)}_2 \quad (4)$$

Infrared Spectroscopy. Figure 1 shows infrared spectra of TosN=IPh in the solid state and in methanol solution. Although some regions of the spectrum are lost due to solvent absorption, the spectra are easily seen to be different. The three most striking changes are the loss of the strong 1265-cm^{-1} band, and the appearance of new bands at 3370 and 1330 cm^{-1} in the transition from the solid phase to the solution phase. Evidently a very polar bond has been eliminated. The new absorption at 1330 cm^{-1} is coincident with the absorption of *p*-toluenesulfonamide in this solvent (CD_3OD). The absorption at 3370 cm^{-1} may be the net absorption difference between O–D and N–D stretching vibrations, since the absorbance is proportional to concentration of TosN= IPh and is in constant ratio to all other bands in the spectrum. Several bands arising from phenyl ring vibrations are retained in the solution spectrum. The spectrum of (dimethoxyiodo)-benzene showed no unique, strong absorbances in the spectral windows of Figure 1.

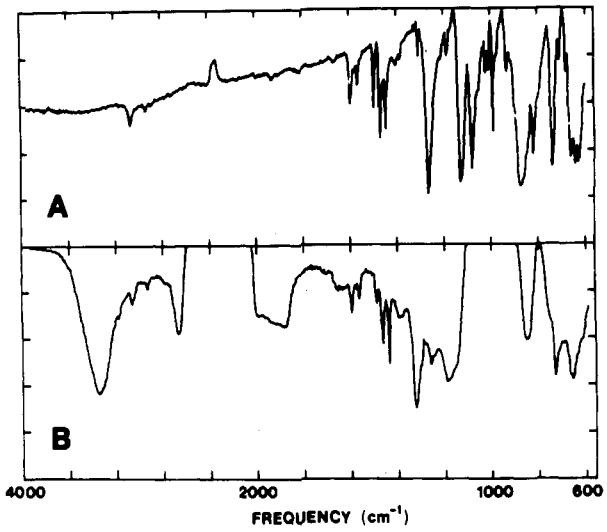

Figure 1. Infrared spectra of TosN=IPh: (A) solid phase in KBr matrix; (B) liquid phase in CD_3OD solution. This solvent provides "windows" that are more useful than those with CH_3OH.

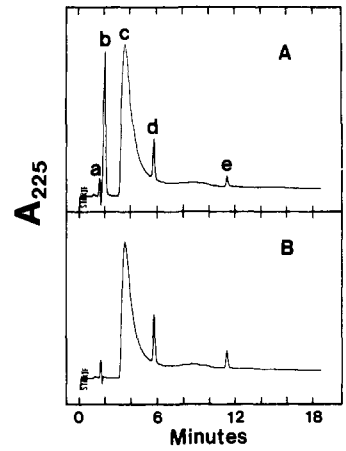

Figure 2. Reverse-phase liquid chromatograms of methanolic solutions of TosN=IPh and of PhI=O. (A) TosN=IPh. Gradient: initial, 70:30 $MeOH:H_2O$; linear gradient to 100% MeOH over 5 min. Peaks: (a) base-line artifact due to $MeOH-H_2O$ gradient; (b) $TosNH_2$; (c) $PhI(OMe)_2$; (d) PhI; (e) base-line artifact. (B) PhI=O.

Liquid Chromatography. Figure 2A shows the reverse-phase liquid chromatogram of a solution of TosN=IPh in aqueous methanol. Three peaks attributable to the sample may be seen, b, c, and d at 1.8, 3.3, and 5.6 min. Peak b had the same retention time as authentic *p*-toluenesulfonamide, while peak d coincided with iodobenzene, an expected contaminant.[9] Figure 2B shows the chromatogram obtained from a methanolic solution of PhI=O. The only important peak is at 3.3 min. The obvious similarity of chromatograms A and B suggested that peak c was (dimethoxyiodo)benzene.

The large peaks in Figure 2 were collected for positive identification. Peak b was isolated as a white solid and identified as *p*-toluenesulfonamide by UV, IR, and NMR spectra. Peak c

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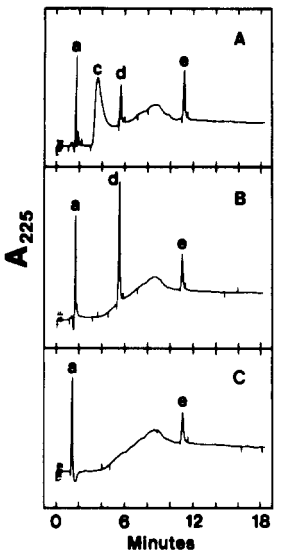

Figure 3. Collection, reinjection, and reduction of peak c. (A) Peak c from the HPLC of TosN=IPh was collected and reinjected. Because of the dilution entailed by reinjection, the recorder attenuation was set lower and the base-line artifacts are more evident than in Figure 2. (B) Peak c was treated with aqueous sodium bisulfite for 5 min prior to reinjection. (C) Blank solvent gradient was run showing the base-line artifacts that are superimposed on runs A and B.

yielded a yellow solid following evaporation of the aqueous methanol HPLC mobile phase. UV, IR, and NMR spectra demonstrated this solid to be iodosylbenzene. We infer that the original compound represented by peak c was (dimethoxyiodo)-benzene, based on the solvolytic behavior of iodosylbenzene reported by Schardt and Hill.[7] However, attempts to isolate the putative (dimethoxyiodo)benzene failed, due to our inability to completely remove water from the aqueous methanol prior to evaporation. Consequently, we were able only to isolate the hydrolysis product, iodosylbenzene. Peak d was shown to be iodobenzene by UV and NMR spectra.

Figure 3A shows a reinjection chromatogram of peak c. Peak b is no longer present, demonstrating that peak c cannot equilibrate with solvent to produce additional *p*-toluenesulfonamide. Figure 3B shows the reinjection chromatogram of peak c after pretreatment with a mild reductant, sodium bisulfite. Peak c completely transforms to peak d (iodobenzene), with no formation of *p*-toluenesulfonamide. The experiments in parts A and B of Figure 3 demonstrate that compound c retains the iodobenzene but not the sulfonamide moiety and cannot be unchanged TosN=IPh. Figure 3C is a solvent blank run, showing background peaks and base-line artifacts that should be subtracted from the chromatograms in Figure 3A,B. Thus, TosN=IPh reacts with methanol to produce *p*-toluenesulfonamide and the same compound as is produced by iodosylbenzene in methanol (i.e., (dimethoxyiodo)benzene). The solvolytic scheme in eq 4 is, then, essentially correct.

Proton Magnetic Resonance Spectroscopy. Additional confirmation of the solvolysis of TosN=IPh was sought by NMR. The proton NMR spectrum of a solution of TosN=IPh in CD_3OD is shown in Figure 4. Of course, in this solvent the methoxy groups of (dimethoxyiodo)benzene will be deuteriated and invisible. A complex multiplet extending from 7.2 to 8.6 ppm represents the superimposition of the (dimethoxyiodo)benzene and *p*-toluenesulfonamide phenyl signals, while the tosyl methyl signal is seen at about 2.5 ppm. Close examination of the 2.5 ppm signal reveals two peaks (2.50 and 2.45 ppm), in the ratio 8.2:1. We considered that the two peaks represented *p*-toluenesulfonamide and intact TosN=IPh. In support of this assignment, we observed that (((phenylsulfonyl)imino)iodo)benzene exhibits no NMR signals in the region 2–3 ppm. However, when *p*-toluenesulfonamide was added to the solution of the phenylsulfonyl derivative, a small δ 2.45 signal developed, in addition to the expected methyl signal at δ 2.50 of *p*-toluenesulfonamide. This experiment shows

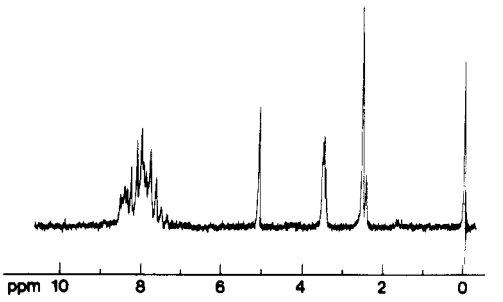

Figure 4. 60-MHz proton NMR spectrum of TosN=IPh in CD_3OD. The multiplet at 3.5 ppm represents residual CHD_2OD while the singlet at 5.1 ppm represents residual CD_3OH.

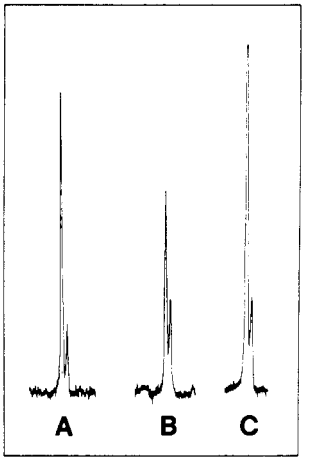

Figure 5. Perturbation of the solvolytic equilibrium. NMR spectra were recorded in the methyl region (2.3–2.8 ppm) in CD_3OD: (A) 0.1 M TosN=IPh; (B) 0.1 M TosN=IPh + 0.1 M PhI=O; (C) 0.1 M TosN=IPh + 0.1 M $TosNH_2$. The NMR probe temperature was 27 °C.

that the δ 2.45 peak is due to the methyl group of TosN=IPh, since neither $PhSO_2N$=IPh nor $TosNH_2$ by themselves exhibit such a peak.

Solvolytic Equilibrium. Figure 5 shows NMR experiments focused on the methyl region. Section A shows the two resonances as observed in a simple solution of TosN=IPh. In section B, an equimolar quantity of solid iodosylbenzene has been added, with a resultant perturbation of the methyl signals, even though iodosylbenzene itself has no resonance in this region. The lower field resonance (*p*-toluenesulfonamide) has been diminished, while the higher field resonance (iminoiodinane) has increased. This evidently results from a reversal of the equilibrium between TosN=IPh and *p*-toluenesulfonamide (eq 4). The added iodosylbenzene solvolyzes to (dimethoxyiodo)benzene, pushing eq 4 toward formation of iminoiodinane. The equilibrium may also be reversed by the addition of *p*-toluenesulfonamide, as shown in part C. Of course, the lower field resonance is now greatly enhanced, but inspection reveals that the higher field resonance is also greater.

The equilibrium expression for eq 4 is shown in eq 5, where K_s is a combined constant which includes the solvent (methanol) concentration.

$$K_s = \frac{[TosNH_2][PhI(OMe)_2]}{[TosN{=}IPh]} \tag{5}$$

When no $TosNH_2$ or $PhI(OMe)_2$ has been added, then $[TosNH_2] = [PhI(OMe)_2]$ and eq 5 may be rewritten as eq 6.

$$\log \frac{[TosNH_2]}{[TosN{=}IPh]} = \log K_s - \log [TosNH_2] \tag{6}$$

A plot of log ($[TosNH_2]/[TosN{=}IPh]$) vs. log $[TosNH_2]$ will be a straight line with slope = −1 and y intercept = log K_s. The concentrations of $TosNH_2$ and of unsolvolyzed TosN=IPh at equilibrium were measured by NMR spectroscopy at several

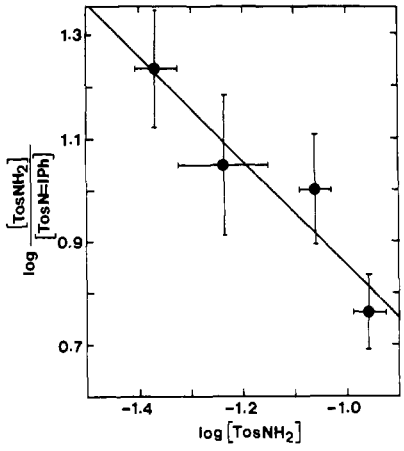

Figure 6. Determination of the solvolytic equilibrium constant by NMR. The concentrations of $TosNH_2$ and of TosN=IPh were determined as described in the Experimental Section. The cross bars represent the standard deviations for the measured quantities. The linear regression line drawn has a slope of −1.012 and a y intercept of −0.1575, with a correlation coefficient of 0.9468.

nominal concentrations of TosN=IPh. The plot is shown in Figure 6. Clearly, the ratio of the tosylamine to the iminoiodinane follows eq 6, increasing as the concentration of tosylamine decreases. The value of K_s indicated by Figure 6 is 0.70 ± 0.10 M. Thus, the NMR experiments reveal that the solvolysis of TosN=IPh is not complete but represents a freely reversible equilibrium. It appears that methanolic solutions of TosN=IPh contain a component of iminoiodinane, with the exact fraction determined by conditions.

Forward and reverse rate constants for eq 4 could not be determined. However, a lower limit was assessed for the forward constant as follows. A nearly saturated solution of TosN=IPh (0.15 M in deuteriated methanol) was rapidly mixed with fresh deuteriated methanol in an NMR tube and the region δ 2–3 scanned as soon as possible. Within the dead time of this experiment (17 s), the equilibration was complete, the ratio $TosN{=}IPh/TosNH_2$ changing from the initial value of 0.18 to the final value of 0.10 predicted by eq 6 for a 2-fold dilution. Additional scans showed no further changes. Assuming that at least five equilibration half-times had occurred within 17 s, then $k_{obsd} \geq 0.20\ s^{-1}$ at 27 °C. A related observation showed that 20 mg of TosN=IPh solvolyzed into 1 mL of methanol within 5 s at 25 °C. Clearly, from these observations the methanolysis of TosN=IPh is fast, and any solution of TosN=IPh that is more than a few seconds old will have completely equilibrated.

Discussion

We have provided substantial evidence in this report that ((tosylimino)iodo)benzene solvolyzes in methanol in an equilibrium process, as depicted in eq 4. The infrared spectrum indicated a substantial change in the covalent structure of the molecules of TosN=IPh. A new band appeared, identical in position with a strong band of *p*-toluenesulfonamide. Liquid chromatography of a methanol solution of TosN=IPh revealed two major components, which were identified as *p*-toluenesulfonamide and (dimethoxyiodo)benzene by comparison with the authentic compounds on the basis of retention times, UV spectra, IR spectra, NMR spectra, and reduction behavior. By means of proton NMR, two components containing the tosyl group were detected and assigned the identities of *p*-toluenesulfonamide (ca. 90%) and (tosylimino)phenyliodinane (ca. 10%). Additional NMR experiments proved that the solvolysis was a fast equilibrium process which could be shifted in either direction by altering concentrations of reactants or products. ((Tosylimino)iodo)benzene was not detected in the HPLC experiment because the solvolysis equilibrium was driven to completion by the continuous supply of fresh solvent in the dynamic chromatographic situation.

These results may explain several reports in the literature of lability of the atom attached to iodine in the oxidation of enzymes by iodine-based oxidants. Heimbrook and Sligar,[1c] Macdonald et al.,[1d] and White and McCarthy[2a] each reported virtually complete incorporation of solvent water into the product alcohol in hydroxylations of various substrates by cytochrome P-450 and iodosylbenzene. Similarly, White and McCarthy[2a] and Murugesan and Hecht[4] reported the production of oxygenated products (alcohols and epoxides, respectively) from oxidations of substrates by TosN=IPh using either cytochrome P-450 or ferric bleomycin. In each case, the products observed are those predicted by solvolysis of the hypervalent iodine compound prior to reaction with the enzyme. In at least one of these cases,[2a] the exchange phenomenon was incorrectly interpreted as due to hydrolysis of an intermediate enzyme iron–nitrene complex. Most recently, Svastits et al.[2b] reported the incorporation of nitrogen into the oxidized substrate using cytochrome P-450 with an analogue of ((tosylimino)iodo)benzene in which intramolecular nitrogen transfer was possible. The aminated product was accompanied by the corresponding alcohol, representing the solvolysis process reported here.

These results should be considered an extension of Schardt and Hill's[7] earlier work on solvolysis of iodosylbenzene. They demonstrate that analogous chemistry pertains to the related iminoiodinane compounds. Whether methanolic solutions of iodosylbenzene contain an appreciable equilibrium concentration of PhI=O is not clear. However, the solvolysis phenomenon must be considered when hypervalent iodine compounds are used as oxidants for enzymes or metalloporphyrins with hydroxylic solvents present. Not only may the kinetics be misleading but also the nature of the proximal oxidant of the enzyme may be very different from that expected.

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