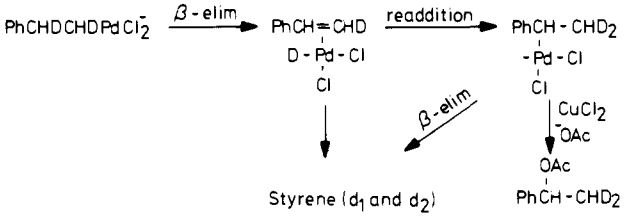
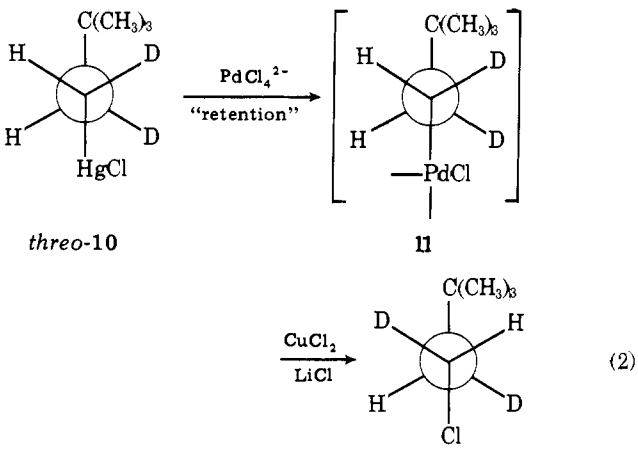
Scheme II



2 indicates that this compound is formed by a β-elimination–readdition sequence similar to that proposed[5b,16] in the Wacker process, followed by cleavage of the palladium–carbon bond by acetate.[9a,17] The expected acetates **5a** from such a sequence are $PhCH(OAc)CHD_2$ and $PhCD(OAc)CH_2D$. The sequence for the formation of the former acetate is given in Scheme II. NMR analysis of the isolated acetate mixture **5a** indicated the presence of a benzyl proton from only one compound, and, more importantly, this proton appears as a doublet.[18] Evidently, the deuterium content is retained in the molecule, which eliminates a path involving acetic acid addition to free styrene.

For comparison we have also studied the stereochemistry of the cupric chloride cleavage of a palladium–carbon bond in one case where participation of any kind is excluded (eq 2).



(2)

The mercury compound *threo*-**10**[19] was used to generate an intermediate palladium compound **11.** Reaction of *threo*-**10,** using the same conditions as those employed above for reaction of **6**, gave the chloride *erythro*-**12.**[20] Since alkyl transfer from mercury to palladium is known[21] to take place with retention, the results show that in this case the cleavage of the palladium–carbon bond has occurred with inversion.

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Proton Nuclear Magnetic Resonance Characterization of the Electronic Structure of Horseradish Peroxidase Compound I

Sir:

The detailed electronic structures of the reactive forms[1] of horseradish peroxidase compound I (HRP-I) and compound II (HRP-II) have remained unresolved despite extensive research in the past few years, largely because of the apparently contradictory evidence from a number of spectroscopic or physical measurements.[2-8] Susceptibility data,[2] as well as Mössbauer[3-5] and EPR[5] studies, support the low-spin (ls) iron(IV) configuration for both the green HRP-I and red HRP-II, which are 2 and 1 oxidizing equiv[1] above the resting enzyme, HRP. The inferred free-radical nature of the second oxidizing equivalent[1] in HRP-I has been attributed to the presence of a porphyrin cation radical based on their optical spectra.[6] The observation of an anomalous ESR signal[5,7] is not inconsistent with this proposal, although a spin-coupled amino acid centered radical, similar to that reported for the cytochrome *c* peroxidase ES complex,[9] has also been suggested.[7]

The recently reported[8] ^1H NMR spectrum of HRP-I, however, has been interpreted as providing strong evidence against a porphyrin cation radical and for a high-spin (hs) iron(IV).[10] We present here new ^1H NMR data on deuterohemin-reconstituted[11] horseradish peroxidase compound I (deutero-HRP-I) which provide an alternative interpretation that is consistent with a ls iron(IV) cation radical formulation for compound I.

Deuterohemin was reconstituted into apo-HRP and purified

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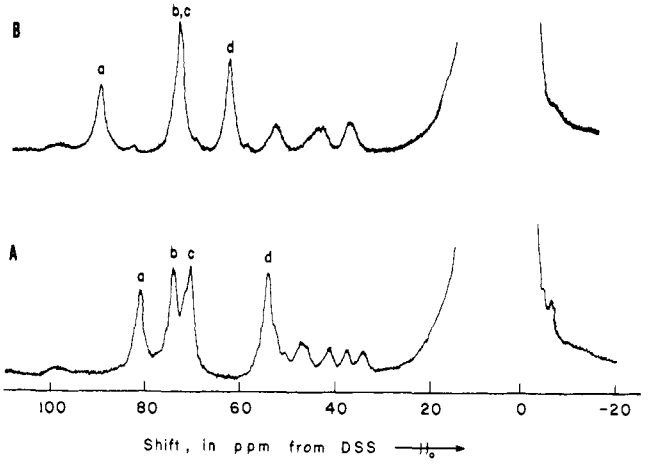

Figure 1. 360-MHz ^1H NMR spectrum of (A) HRP and (B) deutero-HRP. Protein concentration is 2.5–3.0 mM in 0.2 M $NaCl$-^2H_2O, 25 °C, pH 7.0. 2,2-Dimethyl-2-silapentane-5-sulfonate, DDS, is internal calibrant.

as described elsewhere.[11] The 360-MHz ^1H NMR spectra[12] of resting HRP and deutero-HRP are illustrated in Figure 1. As found in other hs ferric hemoproteins,[13] very similar heme methyl (a–d) shift patterns are observed. HRP-I and deutero-HRP-I were prepared by adding an excess of a 0.3 M H_2O_2 solution in ^2H_2O, and their 360-MHz ^1H NMR spectra[13] are shown in Figure 2. The trace of HRP-I is similar to that reported earlier[8] except for improved resolution. The spectrum of deutero-HRP-I exhibits lower signal to noise owing to its much lower stability,[14] but the four heme methyls, a–d, are clearly resolved. The heme 2,4-pyrrole protons, e and f, appear upfield.[15]

The reasonable proposal[8] for hs iron(IV) HRP was made on the basis of similar pyrrole-H shifts for the previously reported hs iron(III)[16] and iron(IV)[10] model complexes and the fact that the heme methyl shifts in HRP and HRP-I are similar (i.e., compare A in Figure 1 and 2). The apparent similarity of the shifts to the hs iron(IV) model[10] was interpreted[8] to exclude the porphyrin cation radical.

However, as shown in B of Figure 2, while the pyrrole-H shifts are considerably downfield (~60 ppm) in hs iron(IV) models,[10] the same functional group in deutero-HRP-I exhibits considerable upfield shifts. Thus the similarity in methyl shifts in HRP-I and hs iron(IV) models is an unfortunate coincidence not recognizable in the spectrum of HRP-I.[8] The large downfield methyl and upfield proton shifts in deutero-HRP-I are consistent with extensive spin delocalization primarily in the π systems,[17] as may be expected for a free radical. The relatively narrow lines observed for both compound I systems cannot themselves be used to argue against[8] the porphyrin cation radical since too little is known about relaxation mechanisms in such strongly coupled spin systems.

While the deutero-HRP-I NMR spectrum does obviate simple hs iron(IV),[10] the hyperfine shifts are only consistent with the porphyrin cation radical; they do not provide any direct evidence for the hypothesis. A porphyrin cation radical, however, is indirectly suggested by a comparison of the methyl line width in HRP-I and deutero-HRP-I (Figure 2). Although the methyl line widths in HRP-I are all very similar, those of deutero-HRP-I are on the average much broader and increase dramatically with shift. It has been proposed that, while both HRP-I and deutero-HRP-I possess porphyrin radical cations, the former[6] exhibits the a_{2u} and the latter[11] the a_{1u} orbital ground state.

Semiempirical molecular orbital calculations indicate[18,19] that, while neither ground state places significant spin density at the pyrrole C_β's, the a_{2u} places the spin density overwhelmingly on the meso positions and a_{1u} puts the spin density primarily on the pyrrole C_α's. These predictions are supported by ESR on porphyrin model compounds.[19] Thus the cation-radical spin density, which is proposed to be closer to the peripheral methyls in the a_{1u} than the a_{2u} ground state, could account for the more effective methyl proton relaxation in deutero-HRP-I.

We therefore conclude that the deutero-HRP-I ^1H NMR spectrum is inconsistent with the simple hs iron(IV)–amino acid radical formulation and that a porphyrin cation radical[6] cannot be excluded at this time. Based on the recent Mössbauer data[5] which support the same ls iron(IV) for both HRP-I and HRP-II, and the previous NMR studies[20] which show that the hyperfine shift pattern is highly characteristic for a given oxidation/spin state, the dramatically different hyperfine shift patterns[8] in HRP-I and HRP-II can also be taken as evidence

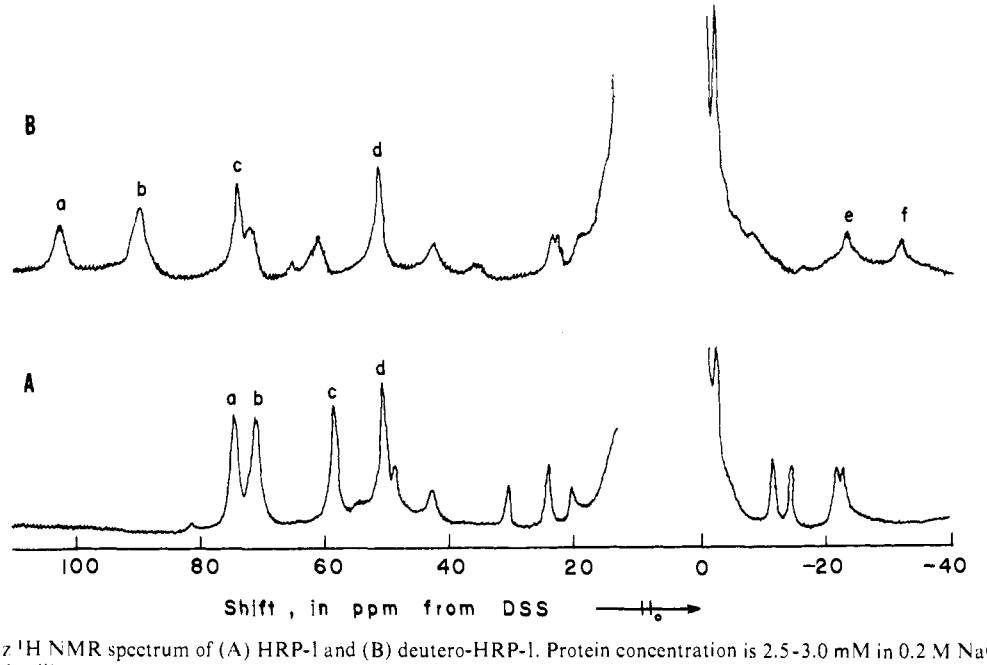

Figure 2. 360-MHz ^1H NMR spectrum of (A) HRP-I and (B) deutero-HRP-I. Protein concentration is 2.5–3.0 mM in 0.2 M NaCl, 25 °C, pH 7.0. DDS is the internal calibrant.

for an additional source of unpaired spin density at the heme periphery, namely the cation radical. Current studies in our laboratory on isotope labeling of the heme are expected to provide a more definitive characterization of the second oxidizing equivalent in HRP-I.

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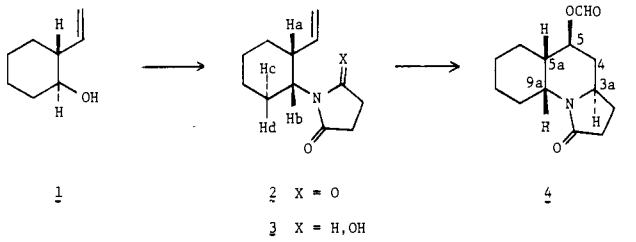
Effect of A$^{(1,3)}$ Strain on the Stereochemical Course of *N*-Acyliminium Ion Cyclizations

Sir:

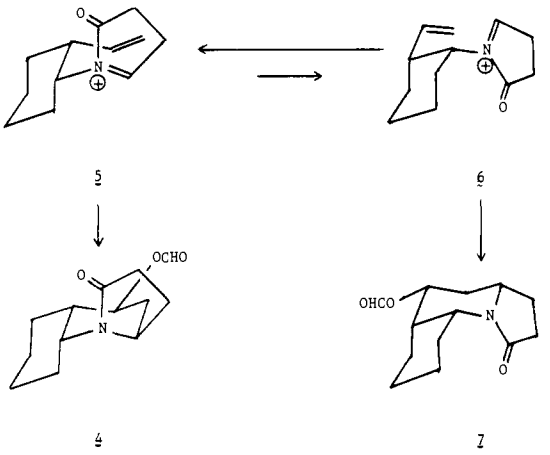
N-Acyliminium ion initiated olefin cyclizations have been documented as a potent tool in alkaloid synthesis.[1] Although a number of stereochemical features of these reactions have been delineated,[2] the effect of asymmetric centers on their stereochemical course has received little attention.[3] Herein are reported results encountered during the course of studies directed toward a synthesis of the *Dendrobatid* alkaloid gephyrotoxin (Scheme I)[4] which illustrate that chiral centers can exert profound influence over the stereochemistry of such cyclizations.

Treatment of *trans*-2-vinylcyclohexanol (**1**)[5] with diethyl azodicarboxylate in the presence of triphenylphosphine and succinimide[6] gave imide **2** (mp 63–66 °C; 50%). Reduction of **2** with diisobutylaluminum hydride[7] afforded carbinolamide

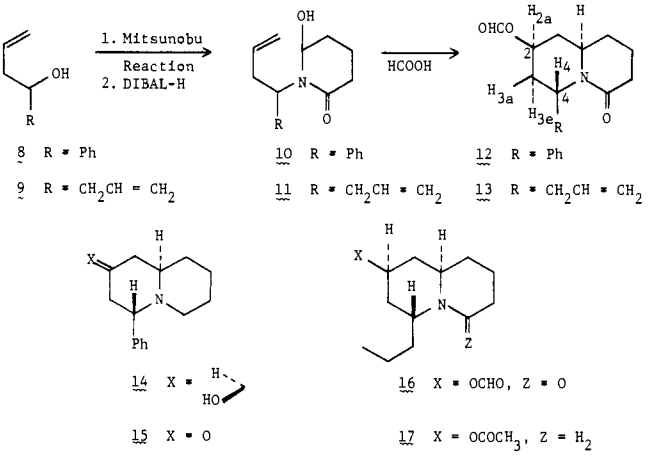
Scheme I



Scheme II



Scheme III



3 as a mixture of diastereomers (mp 93–108 °C; 57%). Treatment of **3** with formic acid (25 °C; 30 min) gave an 85% yield of tricyclic lactam **4** (mp 100–102 °C). The stereochemical assignment for **4** followed from the coupling pattern of the C-5 proton, which appeared as a triplet of doublets (J = 11, 11, 4 Hz) at δ 5.36 ($CDCl_3$).[8] Of the four possible *cis*-decahydroquinolines which could have resulted from the *N*-acyliminium ion cyclization, only **4** can adopt a conformation in which the C-5 proton affords two anti and one gauche coupling to protons at C-4 and C-5a.[9]

Two factors may be responsible for the stereoselective conversion of **3** into **4.** The cyclization of **3** most likely proceeds through an *N*-acyliminium ion which can adopt chair–chair conformations **5** and **6** (Scheme II). ^{1}H NMR analysis indicates that imide **2** adopts a chair conformation in which the vinyl group occupies an axial site (J_{ab} = 4, J_{bc} = 12, J_{bd} = 4 Hz). This suggests that **5** represents the most stable conformation of the *N*-acyliminium ion. In addition to the ground-state energy difference between the conformations leading to **4** and its C-3a,5 isomer **7,** it is probable that the E_{act} for conversion of **6** into **7** is greater than that for conversion of **5** into

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