states has strongly indicated the presence of a thiolate ligand to the heme iron. Of the reduced + CO model complexes prepared by Collman and Sorrell,[9g] only the MCD spectrum[7] of the thiolate model matched that of reduced + CO P-450$_{\text{LM2}}$, reproducing each spectral attribute from the single visible band centered at 560 nm to the shoulder at 420 nm and the "extra" negative feature at 370 nm. Other models[7] showed substantial differences throughout the spectral region examined including distinct α and β bands in the visible region and lack of the "extra" negative band at 370 nm. Our work with oxidized high-spin P-450 model complexes, presented in the preceding communication,[6] provides additional spectral evidence for thiolate ligation. From that work we concluded that the appearance of a strong negative MCD band in the 395-nm Soret region would be diagnostic for thiolate ligation in high-spin ferric heme complexes. All of these results, in conjunction with the aforementioned similarity between the MCD spectra of CPO and P-450, provide strong evidence for thiolate ligation of the heme iron of CPO in its oxidized and reduced + CO states. It should be emphasized, however, that these results are based on physical measurements, whereas the contrary conclusion,[8] that a sulfur derived ligand is not present, was based on chemical studies of CPO. It is possible that the inability of Chiang et al.[8] to detect chemically a free sulfhydryl group in the native enzyme may be due to its hidden nature within the protein. To explain the lack of a free sulfhydryl group in the denatured enzyme, one needs to postulate that the thiolate ligand of CPO, when released from the iron, is sufficiently activated to react with the only other half-cystine residue in CPO[22] to form a disulfide bond. Such an explanation, if true, would remove the apparent discrepancy between chemical[8] and spectroscopic results.

The present work offers another illustration of the power and utility of MCD spectroscopy in defining certain structural aspects of this biologically important class of heme proteins.

Acknowledgments. We wish to thank Ruth Records for running some of the MCD spectra, Dr. Larry Vickery (University of California, Berkeley) for permission to replot data,[13b] and the National Institutes of Health (Grants GM 20276-02 and RG 7768) and the National Science Foundation (Grant No. B MS71-01280C) for financial assistance. One of us (J.H.D.) wishes to thank Dr. D. A. Haugen and Professor M. J. Coon for helpful discussions concerning the isolation of mammalian P-450$_{\text{LM2}}$.

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CIDNP from Grignard Reagents Undergoing Iron-Catalyzed Halogen–Metal Exchange

Sir:

Recent reports[1] of CIDNP from Grignard reagents formed by the reaction of organohalides with magnesium have revived the view that free radicals are key intermediates in the synthesis of organomagnesium compounds.[2] While there can be no doubt that free radicals are readily formed in solutions of Grignard reagents under a variety of conditions,[2,3] the observation of CIDNP from the reagent itself has been considered to be strong evidence implicating radicals in the *primary* step of formation.[1,4]

We report here an observation which suggests an alternative interpretation of the CIDNP experiments: CIDNP of the reported type may also be produced in Grignard reagents during their iron-catalyzed reactions with organohalides *in the absence of metallic magnesium.*

In Figure 1 is shown the 0 to −1 ppm region of the proton NMR spectrum obtained before, during, and after the reaction of 0.6 M isopropylmagnesium bromide with 1.2 M *n*-butyl bromide in THF catalyzed by the addition of 10^{-4} M $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$. The spectra show clearly that (a) *n*-butylmagnesium bromide is formed by halogen–metal exchange with isopropyl Grignard and, more importantly, (b) at least some of the *n*-butyl Grignard is formed via the intermediacy of *n*-butyl radicals as evidenced by the appearance of E/A CIDNP in the

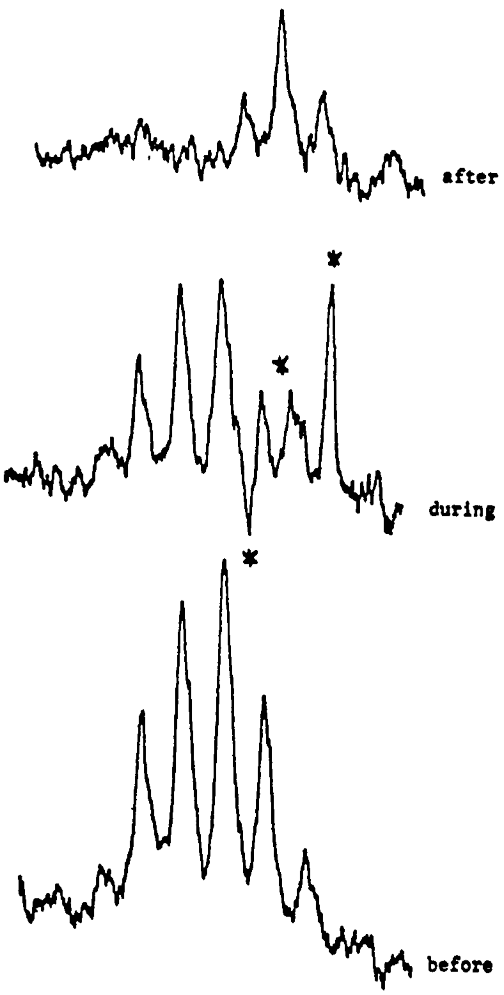

Figure 1. NMR spectrum in region from 0 to -1 ppm before, during, and after reaction of an 0.6 M THF solution of $(CH_3)_2CHMgBr$ with 1.2 M $CH_3(CH_2)_3Br$ in the presence of 10^{-4} M $FeCl_2$ at 37 °C. Lines from $CH_3(CH_2)_3MgBr$ exhibiting CIDNP are starred in the center spectrum.

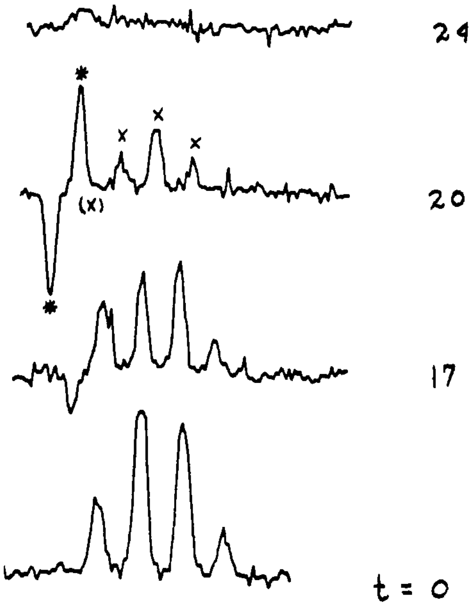

Figure 2. NMR spectrum in region from 0 to -1 ppm scanned at various times after the beginning of the reaction between 0.8 M CH_3CH_2MgBr, 1.4 M $(CH_3)_2CHCH_2I$, and 10^{-4} M $FeCl_2{\cdot}4H_2O$ in THF at 37°. The time of each scan after addition of $FeCl_2$ to the solution is indicated on the right. In the scan taken at 20 s the lines arising from $(CH_3)_2CHCH_2MgX$ are indicated with stars (*) and those from CH_3CH_2MgX with (×).

triplet from the α-protons of the reagent. As shown in Figure 2, weak polarization may also be observed in the protons of the Grignard reagent which is initially present.[5] Weak dehancement of one of the two middle lines in the quartet from CH_3CH_2MgBr (0.8 M) is observed during its reaction with isobutyl iodide (1.4 M) catalyzed by $FeCl_2{\cdot}4H_2O$ (10^{-4} M). The samples in Figures 1 and 2 also exhibit strongly enhanced NMR lines at lower field due to alkane and alkene coupling and disproportionation products, as reported previously.[3a,b,7]

The above observations are consistent with the following scheme for reactions between an organohalide, RX, and a Grignard reagent, R′MgY in the presence of catalytic amounts of an iron salt.

$$RX \xrightarrow[\text{catalyst}]{\text{R}'\text{MgY and}} R\cdot \tag{1}$$

$$R\cdot + R\cdot \xrightarrow[\text{encounters}]{\text{diffusive}} \underset{\mathbf{1}}{\overline{R\cdot \quad \cdot R}} \tag{2}$$

$$\mathbf{1} \xrightarrow{\text{combination}} \text{alkanes, alkenes} \tag{3a}$$

$$\mathbf{1} \xrightarrow[\text{R}'\text{MgY}]{\text{scavenging}} R\ MgY + R'\cdot \tag{3b}$$

The phase of the alkyl Grignard reagent CIDNP (E/A) is the same as that observed in the formation reaction with magnesium metal[1] and is opposite that observed from the alkane and alkene combination products. This is just what is expected for a scavenging product formed from a diffusive radical encounter pair, **1**, by -MgY transfer from a molecule of Grignard reagent (eq 3b). The CIDNP results thus confirm the suggestion made many years ago[8] that a homolytic pathway exists for halogen-metal exchange involving Grignards. The observation of CIDNP, of course, does not rule out parallel, nonradical pathways for halogen-metal exchange. Indeed, attempts by ourselves[9] and others[10] to detect analogous NMR enhancements in alkyllithiums undergoing halogen-metal exchange have met with no success at all, despite the strong CIDNP observed from coupling products formed concurrently.[9,11]

It has recently been shown[12] that small amounts of oxidizing impurities in combination with the trace impurities native to magnesium of the grade employed by Bickelhaupt et al.[1] exhibit catalytic activity in the reaction of an alkyl halide with its Grignard reagent equivalent to added $FeCl_2$. Furthermore, CIDNP observed during the metal-catalyzed reaction is detected exclusively in products derived from the alkyl halide,[3a] presumably via selective formation of radicals from that source. This implies that enhancement should appear primarily in the newly formed Grignard reagent, as observed both in Figures 1 and 2 and, of course, during the reaction with metallic Mg.[1] Thus catalyzed halogen-metal exchange of newly formed Grignard reagent with alkyl halide during the reaction of an alkyl halide with magnesium cannot be ignored. The *extent* to which the exchange process is responsible for the observed Grignard reagent polarization is not yet known with certainty, although an argument can be made that it is minor.[13] Conclusive proof of the intermediacy of radical pairs in the mechanism of formation of Grignard reagents, however, must await the discovery of conditions under which the contributions to the radical flux made by the Grignard reagent before and after its formation can be distinguished.

Acknowledgments. We are grateful to the National Science Foundation for financial support. Our thanks go also to Professor F. Bickelhaupt for discussion of the CIDNP experiments from both our laboratories.

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On the Origin of CIDNP Observed during the Grignard Formation Reaction

Sir:

In a series of papers[1] we have proposed that the formation of Grignard reagents from alkyl halides and metallic magnesium occurs essentially by the reaction of radicals, R·, with surface bound (subscript "s") magnesium halide $\cdot XMg_s$ under the influence of base (eq 1, 2, 3, 4 and 4′):

$$RX + Mg \rightarrow RX\cdot^- + Mg_s^+ \tag{1}$$

$$RX\cdot^- + Mg_s^+ \rightarrow R\cdot + \cdot XMg_s \tag{2}$$

$$R\cdot + R\cdot \rightarrow \overline{R\cdot\cdot R} \rightarrow 2{*}R\cdot \tag{3}$$

$$R\cdot + \cdot XMg_s \xrightarrow{\text{base}} RMgX \tag{4}$$

$${*}R\cdot + \cdot XMg_s \xrightarrow{\text{base}} {*}RMgX \tag{4'}$$

The solvent dependence of CIDNP and product yields,[1b,c,e] as well as the observation of CIDNP in RMgX itself,[1a,b] were the major evidence for this scheme. They also made it reasonable and attractive to suggest a common reaction step (eq 4 and 4′) leading to both polarized and nonpolarized Grignard compounds.

Recently Lawler and Livant[2] reported the observation of CIDNP in Grignard compounds during metal–halogen exchange reactions in THF, catalyzed by the addition of 10^{-4} M $FeCl_2 \cdot 4H_2O$, rationalizing their results in the following way:

$$RX \underset{\text{catalyst}}{\xrightarrow{R'MgY\text{ and}}} R\cdot \tag{5}$$

$$R\cdot + R\cdot \underset{\text{encounters}}{\xrightarrow{\text{diffusive}}} \underset{\mathbf{1}}{\overline{R\cdot\cdot R}} \tag{6}$$

$$\mathbf{1} \xrightarrow{\text{combination}} \text{alkanes, alkenes} \tag{7}$$

$$\mathbf{1} \underset{R'MgY}{\xrightarrow{\text{scavenging}}} RMgY + R'\cdot \tag{8}$$

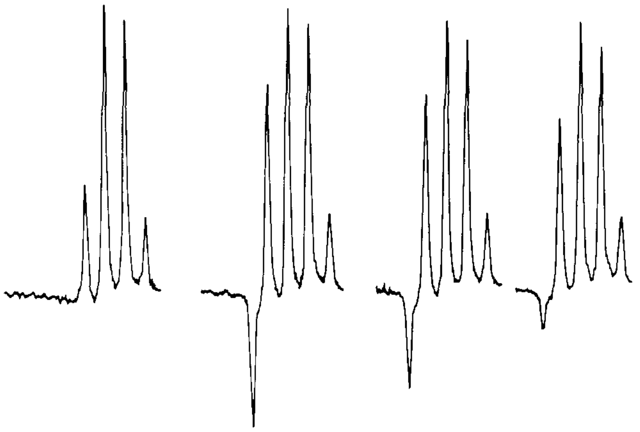

Figure 1. The 60-MHz spectrum in region from 0 to −1 ppm (relative to Me_4Si) before and during the reaction of 0.33 M $(CH_3)_2CHCH_2I$ with magnesium (12 mg) in the presence of 1 M CH_3CH_2MgBr in THF (0.5 ml) at 40°.

They suggested that their results made it likely that CIDNP, observed in our experiments, in the Grignard formation reaction was not due to radicals originating from sequence eq 1–4 but from the reactions given in eq 5–8. This suggestion was based on the idea that small amounts of oxidizing impurities in combination with trace impurities native to magnesium of the grade employed in our laboratory[3] exhibit catalytic activity in the reaction of an alkyl halide with its Grignard reagent, equivalent to added $FeCl_2$.

We wish to present additional evidence which proves that neither are the radicals, responsible for CIDNP in the Grignard formation reaction, formed by process 5, nor do they—to any observable extent—react according to eq 8.

We observed no reaction at all when preformed Grignard reagent is mixed with alkyl halide in diethyl ether or in di-*n*-butyl ether. In THF also no reaction was observed between an alkyl bromide and a Grignard compound. Only on addition of an alkyl iodide (4.5 M) to a Grignard compound in THF (3 M), very weak polarization could be detected in the starting Grignard; polarization in the newly formed Grignard (eq 8) was either absent or too weak to be conclusively observable. With alkyl iodide slow metal–halogen exchange and Wurtz-type coupling were observed.[4] Concentration—varied between 0.5 and 3–4 M in both reactants—was of no influence on these phenomena.

The difficulty to observe the elusive and weak polarization under these conditions[2] is in sharp contrast with the ease with which CIDNP is observed in the Grignard formation reaction. This could be even more dramatically demonstrated by addition of isobutyl iodide (1 M) to a solution of ethylmagnesium bromide (1 M) in THF containing magnesium metal (Figure 1). In contrast to the observation made by Lawler (ref 2, Figure 2) a pure multiplet spectrum (E/A) was observed for the newly formed isobutylmagnesium iodide only, the spectrum of ethylmagnesium bromide remaining unchanged; this implies that ethyl radicals (R′ in eq 8) were not involved in radical pair formation and that combination of polarized radicals, resulting from eq 2 and 3 with ·MgX is the predominant pathway to