Unambiguous evidence for the above assignment was obtained in the photosensitized decomposition of diazomethane. With benzophenone (10%) as sensitizer[17] the signal of **1** appeared in enhanced absorption (Figure 1a). This alteration of polarization[18] supports the evidence found in the direct photolysis, that the divalent carbon intermediate, methylene, is produced in the singlet state and that it reacts with the carbon–chlorine bonds of tetrachloromethane before intersystem crossing to the triplet ground state[4] can occur.

An important postulate is implicit in this conclusion. According to the theory of CIDNP,[9-11] any polarization of in-cage recombination products vanishes unless there is escape of radicals from their primary encounter cages. The free-radical chain reaction (Scheme I) demonstrates that some of the chloromethyl–trichloromethyl radical pairs separate, and the high quantum yield of this reaction indicates that a sizable fraction of these radicals initiate radical chains. Since it was shown above that the radical pairs are of singlet spin multiplicity, we conclude that *singlet methylene* is involved in the *abstraction* that initiates the chain reaction.

The results discussed above provide an interesting contrast to the mechanisms derived for the reaction of methylene with carbon–hydrogen bonds. The almost indiscriminate insertions into primary, secondary, and tertiary bonds in the liquid phase[19,20] were rationalized as "one-step-insertion" reactions of singlet methylene,[20,21] whereas the more selective "abstraction" reactions in the vapor phase were ascribed to the triplet spin multiplicity.[20,21] The mechanisms postulated for the reactions with the carbon–hydrogen and the carbon–chlorine bonds are *not* mutually exclusive, because the two types of bonds are considerably different.

A short comment on the enhanced signals of **2**, **3**, and **4** follows. The polarization of **2** and **3** is not altered upon the use of a triplet sensitizer, suggesting random recombination of radicals such as chloromethyl with trichloroethyl (A′ + $\cdot CH_2Cl$ → **2**) or trichloropropyl radicals (B′ + $\cdot CH_2Cl$ → **3**). This mechanism leads one to expect identical polarization for all four (six) protons of **2** (**3**) because two of them originate in the α position of one radical fragment ($\cdot CH_2Cl$) and the two (four) others in the β position of the other one (A′, B′). Making the reasonable assumption that A′ (B′) has a larger g value than the chloromethyl radical, one predicts enhanced absorption for both **2** (as is observed) and **3** (contrary to the experimental result). This discrepancy casts some doubt on the assumed mechanism.

(17) The use of benzophenone as a triplet sensitizer in hydrocarbons is limited because it undergoes photoreductions *via* hydrogen abstraction.[9b] This problem does not exist in chlorinated solvents, because the oxygen–chlorine bond is not very attractive.

(18) A similar alteration of an enhanced signal upon triplet photosensitization was found in the decomposition of dibenzoyl peroxide: (a) A. M. Trozzolo, S. R. Fahrenholtz, and F. Heatley, Abstracts, 159th National Meeting of the American Chemical Society, Houston, Texas, Feb 1970, No. ORGN 042; (b) R. Kaptein, J. A. Den Hollander, D. Antheunis, and L. J. Oosterhoff, *Chem. Commun.*, 1687 (1970); (c) S. R. Fahrenholtz and A. M. Trozzolo, *J. Amer. Chem. Soc.*, **93**, 251 (1971).

(19) W. v. E. Doering, R. G. Buttery, R. G. Laughlin, and N. Chaudhuri, *ibid.*, **78**, 3225 (1956).

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(21) *Cf.* H. M. Frey and R. Walsh, *J. Chem. Soc. A*, 2115, (1970), and references cited therein.

The reaction mechanism leading to **4**, the unique eight-step sequence induced by a trichloromethyl radical, cannot explain any polarization of this product,[9-11] yet an enhanced signal is observed both upon direct irradiation (A), and when the triplet sensitizer is used (E). This result can be explained if **4** is formed *via* the competing reaction sequence

$$3 + :CH_2 \longrightarrow [C' + \cdot CH_2Cl] \longrightarrow 4$$

and $\cdot CH_2Cl$ (one α-Cl atom) has a larger g factor than C′ (three β-Cl atoms).

Alternatively, one can account for the high-field signals of Figure 1a and 1c by assigning them to small amounts of 1,2-dichloroethane, which has a chemical shift very close to that of **4**. The dichloroethane could be formed by reactions of chloromethyl radicals[22] which escape from their geminate counterradicals after a polarizing encounter. As mentioned above, such an escape is necessary if any polarization of in-cage products occurs.

At the present time, we are extending our studies to the generation of methylene and the chloromethyl radical from alternative sources and to the reactions of diazomethane with substrates such as **1**, **2**, and **3**.

Acknowledgments. We are grateful to Dr. Saul Meiboom and Mr. Richard Hewitt for their patient guidance during our use of a modified pmr spectrometer and we are indebted to Dr. Sivert H. Glarum for enlightening discussions.

(22) Possible pathways to 1,2-dichloroethane include the induced decomposition of diazomethane (i) followed by radical transfer (ii). Step i should be diffusion controlled, whereas reaction ii should occur in the first few collisions of the 2-chloroethyl radical. Neither reaction i nor ii is acceptable as a polarization step.[9-11] However, it is reasonable that the polarization of chloromethyl radicals escaping from trichloromethyl–chloromethyl radical pairs is in part preserved through a diffusion-controlled reaction with a substrate of 0.1 M concentration.

$$\cdot CH_2\text{-}Cl + CH_2N_2 \longrightarrow \cdot CH_2\text{-}CH_2Cl + N_2 \quad \text{(i)}$$

$$\cdot CH_2\text{-}CH_2\text{-}Cl + CCl_4 \longrightarrow Cl\text{-}CH_2\text{-}CH_2\text{-}Cl + \cdot CCl_3 \quad \text{(ii)}$$

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The Stereochemistry of Reactions Occurring at Iron–Carbon σ Bonds[1]

Sir:

An interest in the mechanisms of transformations involving carbon–transition metal σ bonds has led us to determine the stereochemistry of the reactions between *threo*-$(CH_3)_3CCHDCHDFe(CO)_2Cp$ (**1**) and molecular bromine, mercuric chloride, and sulfur dioxide, using an nmr procedure described previously.[2,3] This procedure depends on the fact that erythro and threo diastereomers of most substances having the composition $(CH_3)_3CCHDCHDX$ display distinct nmr spectra, reflecting preferred molecular conformations in which the bulky *tert*-butyl and X groups are trans

(1) This work was supported by the National Science Foundation, Grant No. GP-14247, and by the National Institutes of Health, Grant No. GM 16020.

(2) G. M. Whitesides and D. J. Boschetto, *J. Amer. Chem. Soc.*, **91**, 4313 (1969).

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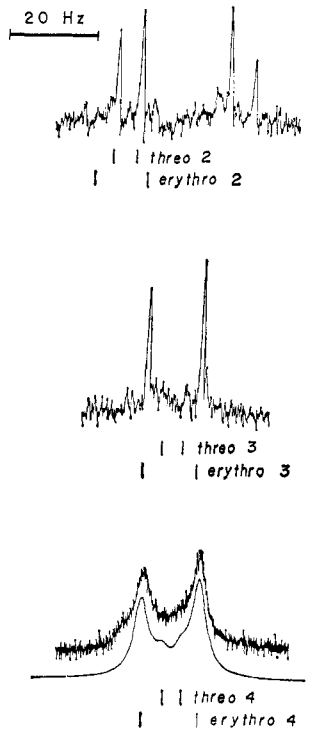
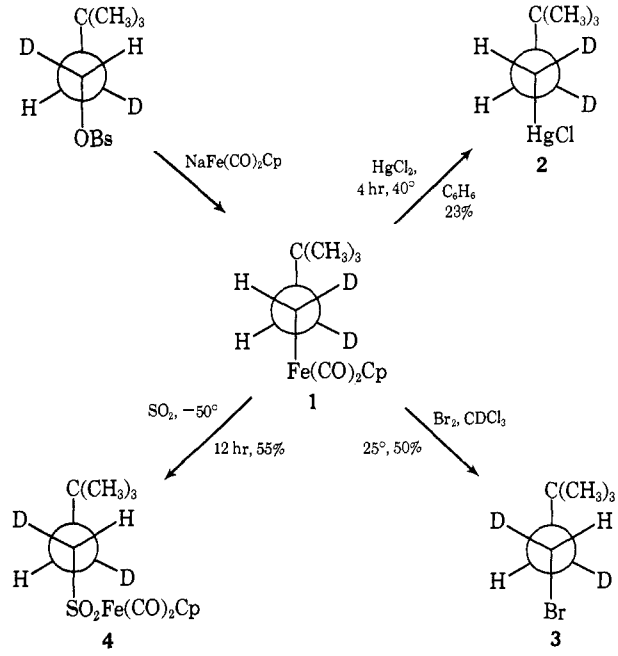


Figure 1. Deuterium-decoupled 100-MHz nmr spectra of the C*H*DC*H*D protons of **2**, the C*H*DBr proton of **3**, and the C*H*DSO_2 proton of **4**. For comparison, the positions of lines characterizing the threo and erythro diastereomers of **2**, **3**, and **4** are given below the traces. The calculated spectrum given for **4** is that expected for a mixture of 82% erythro and 18% threo diastereomers.

across the ethylene moiety.[4] Since the resonances due to threo and erythro diastereomers can be identified directly on the basis of the magnitude of their vicinal C*H*DC*H*D coupling constants,[4] this technique suffers from none of the ambiguities which presently attend efforts to use optical activity to examine the stereochemistry of reactions at carbon–metal bonds. Values of the vicinal coupling constants required for analysis of mixtures of threo- and erythro-**4** were obtained by analysis of the AA'XX' spectrum of $(CH_3)_3CCH_2$-$CH_2SO_2Fe(CO)_2Cp$ [*Anal.* Calcd for $C_{13}H_{18}O_4FeS$: C, 47.87; H, 5.56; Fe, 17.12; S, 9.83. Found: C, 47.99; H, 5.58; Fe, 17.10; S, 8.92; ir (Nujol) 2057, 2007, 1189, 1178, 1048 cm^{-1}]: J_{threo} = 4.3 Hz; $J_{erythro}$ = 13.0 Hz. Analogous coupling constants for the diastereomers of 3,3-dimethylbutyl-*1,2-d*$_2$ mercuric chloride (**2**) and bromide (**3**) can be inferred from data reported previously.[4]

Reaction between **1** and a suspension of mercuric chloride[5] yielded **2**. Isolation of **2**, followed by examination of its deuterium-decoupled nmr spectrum (Figure 1), established that the conversion of **1** to **2** takes place with >90% retention of configuration at carbon.[6] In contrast, analogous experiments demonstrated that the transformation of **1** to **3** on treatment with bromine occurred with high (>90%)[6] stereoselectivity with *inversion* of configuration. Reaction between **1** and sulfur dioxide yielded **4**.[7] The nmr spectrum of this substance was less easily analyzed than those of **2** or **3**, since its lines were relatively broad. However, comparison of the experimental spectrum with spectra calculated for mixtures containing known proportions of *threo-* and *erythro*-**4** indicated that reaction had occurred with approximately 80% *inversion* of configuration.

The stereochemical outcome of the transformation **1** → **2** is the result expected for frontside electrophilic attack[8] of mercuric chloride on the C–Fe bond. Bromination of carbon–metal bonds has been observed to proceed with inversion of configuration in both main[9]



and transition[10] group compounds, although other stereochemical results have also been established.[8,11] Little is known on which to base a mechanistic proposal for the unexpected stereochemistry characterizing the reaction between sulfur dioxide and **1**; however, the report[12] that reaction between organocobalt compounds and sulfur dioxide appears to be catalyzed by water suggests that carbon monoxide insertion[2] may not provide a useful model for this process. Thus, this and previous[2] studies of the stereochemistry of reactions of **1** indicate that both retention and inversion of configuration at carbon may accompany reactions that formally involve both cleavage of carbon–iron σ bonds by electrophilic reagents ($HgCl_2$, Br_2) and "insertion" into carbon–iron bonds by neutral molecules (CO, SO_2).

Oxidative addition of optically active CH_3CHBr-$CO_2C_2H_5$ (**5**) to $Ir(CO)Cl[P(C_6H_5)_2CH_3]_2$ has recently been proposed to occur with retention of configuration, on the basis of the observation that the **5** obtained on

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(8) F. R. Jensen and B. Rickborn, "Electrophilic Substitution of Organomercurials," McGraw-Hill, New York, N. Y., 1968, p 86; D. S. Matteson, *Organometal. Chem. Rev. A*, **4**, 263 (1969).

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cleavage of the intermediate alkyliridium(III) complex with bromine had the same configuration as the starting **5**, and on the *assumption* that bromination of this alkyliridium complex occurred with *retention* of configuration at carbon.[13,14] The *inversion* of configuration established for bromination of **1** indicates that this proposal should presently be accepted with reservation. Although the stereochemistry assumed for the bromination leading to **5** may ultimately be demonstrated to be correct, it is clear that either inversion or retention of configuration may characterize brominative cleavage of carbon–metal bonds.

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(14) Inversion of configuration had been established in oxidative addition of *trans*-1-bromo-2-fluorocyclohexane to $Ir(CO)Cl[P(CH_3)_3]_2$: J. A. Labinger, R. J. Braus, D. Dolphin, and J. A. Osborn, *Chem. Commun.*, 612 (1970).

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Amino Acid Sequence of the Subunits of Ovine Pituitary Interstitial Cell-Stimulating Hormone

Sir:

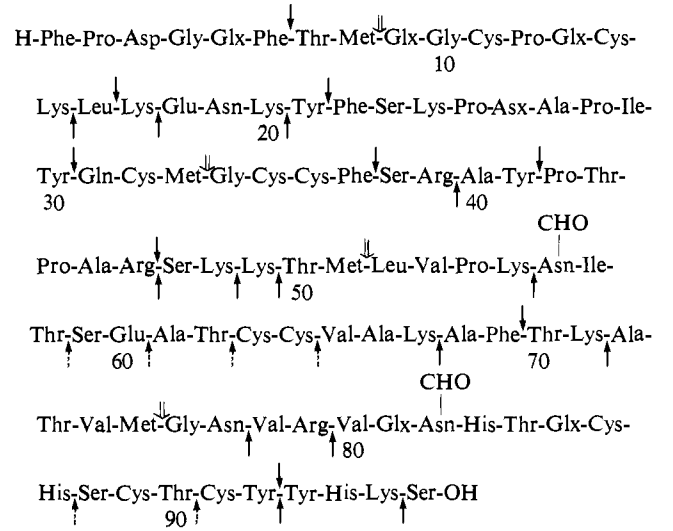
Ovine interstitial cell-stimulating hormone (ICSH, LH), a glycoprotein of about 30,000 molecular weight,[1,2] is a dimer[3] in neutral solutions. Carboxyl terminal group analysis by Ward, *et al.*,[4] suggested the presence of two nonidentical polypeptide chains. Subsequently, by virtue of their isolation by countercurrent distribution,[5] the subunits of ICSH were unequivocally demonstrated to be chemically dissimilar both with respect to amino acid and carbohydrate content. The individual subunits, ICSH-α and ICSH-β (formerly designated ICSH-CI and ICSH-CII), are biologically inactive until recombined.[6–8] Enzyme digestion with carboxypeptidase A showed[9] that the COOH terminal residue of ICSH-α was serine and that of ICSH-β was leucine. More recently, reinvestigation of the N terminus by the dansyl procedure[10] shows that ICSH-α has an NH_2 terminal phenylalanine and ICSH-β begins with serine. We have previously reported on the sequences of two peptides obtained by CNBr cleavage of ICSH-β which accounted for 39 of the 120 residues present[11] in ICSH-β. We wish now to report the results of sequence studies which allow us to postulate the entire linear amino acid sequence of both ICSH-α and ICSH-β.

Ovine ICSH, and its subunits ICSH-α and ICSH-β, were prepared by the procedures previously described.[5,12] Performic acid oxidations as well as reduction and alkylation of the hormone were performed by methods[13,14] reported earlier. CNBr reactions[15] were carried out in 70% formic acid. Completeness of performic acid oxidation, reduction, and alkylation and CNBr cleavage was determined by amino acid analysis of the products. Amino acid analyses[16] were performed with a Beckman amino acid analyzer Model 120 B.

Digestions with trypsin, chymotrypsin, and subtilisin were carried out in 0.1 *M* ammonium acetate of pH 8.3 for 8 hr at 37° (enzyme–substrate, 1:100 or 1:50). CNBr reaction mixtures were fractionated on columns of Sephadex G-50 in 20% formic acid (v/v). Enzyme digests were fractionated first on columns of Sephadex G-50, -25, and -15 in 0.01 *M* NH_4OH; further purifications were effected by paper chromatography in the system 1-butanol–acetic acid–water (4:1:5, v/v), or 1-butanol–pyridine–acetic acid–water (5:2:1:4, v/v) and by high- and low-voltage electrophoresis on paper in buffers of either pH 2.0 or 6.4. Purity of peptides was assessed by terminal group analysis,[10] quantitative amino acid analysis,[16] and by the fingerprint technique.[17] Sequences were established by the Edman[18]–dansyl[10] technique, the Edman subtractive method,[19] and kinetic studies of digestion with leucineaminopeptidase and carboxypeptidase.

The proposed structure of ICSH-α is shown in Chart I and that of ICSH-β in Chart II. ICSH-α consists

Chart I. Amino Acid Sequence of Ovine ICSH-α(CI)[a]



[a] CHO, carbohydrate moiety; ⇓, CNBr; ↑, trypsin; ↓, chymotrypsin; ↑, subtilisin.

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