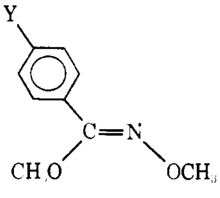
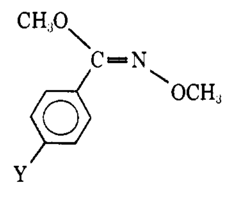


4a, Y = H
b, Y = NO$_2$



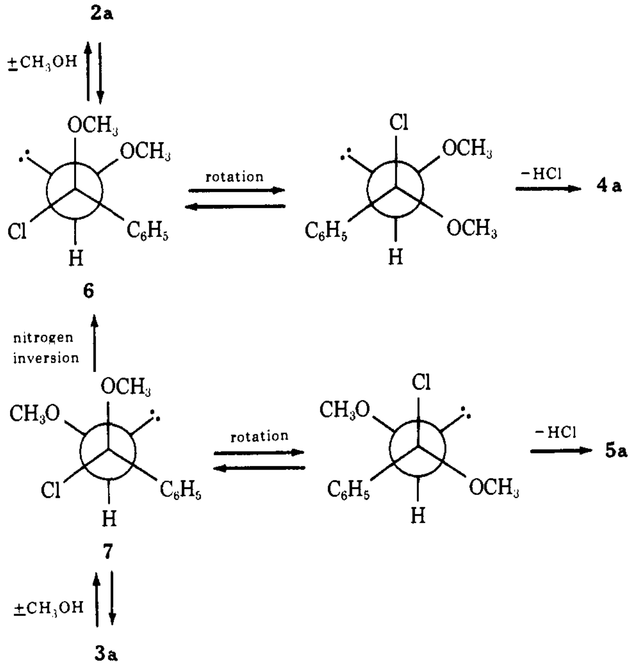
5a, Y = H
b, Y = NO$_2$

hydroximate whose configurations are known.[4] Comparable results were obtained when the *p*-nitrohydroximoyl chlorides, **2b** and **3b**, were allowed to react with sodium methoxide. Compound **2b** produced **4b** (**4b** : **5b** = 98 : 2) whereas **3b** gave mainly **5b** (**4b** : **5b** = 16 : 84).

Further investigation of the (*Z*)-hydroximoyl chloride (**3a**) reaction revealed that the lower stereoselectivity of this reaction is due in part to a methoxide induced isomerization of **3a** to **2a**. The hydroximates **4a** and **5a** were found to be stable under the reaction conditions; no isomerization of these compounds could be detected by glc over a 48-hr period. When the reaction of **3a** with methoxide was quenched with water after approximately one half-life, it was found by glc analysis that some isomerization of **3a** to **2a** had occurred (ratio of **3a** to **2a** after approximately one half-life = 87 : 13). That this isomerization is catalyzed by methoxide ion was demonstrated by the fact that an 0.08 *M* solution of **3a** in DMSO–methanol did not undergo any detectable isomerization at 44.6° over a 48-hr period.

The rates of the hydroximoyl chloride–sodium methoxide reactions were followed by quenching the reactions with water and titrating. Both **2a** and **3a** follow second-order kinetics through at least 60–70% of the reaction. The rate constant for the reaction of **2a** (8.02×10^{-2} *M*) with sodium methoxide (2.88×10^{-2} *M*) was found to be 1.27×10^{-2} l./(mol sec) at 44.6°. At the same temperature, **3a** (7.78×10^{-2} *M*) reacted with sodium methoxide (2.67×10^{-2} *M*) at a rate of 1.44×10^{-2} l./(mol sec). In order to determine the effect of the leaving group on the rate of this reaction, *O*-methylbenzohydroximoyl bromide (**2c**, assumed to have the *E* configuration) was synthesized in low yield by the reaction of **1a** with phosphorus tribromide. The second-order rate constant for the reaction of **2c** (8.06×10^{-2} *M*) with sodium methoxide (2.47×10^{-2} *M*) is only 1.9 times faster than that of the (*E*)-hydroximoyl chloride (**2a**)–sodium methoxide reaction.

Stereoselective inversion of configuration during nucleophilic substitution at sp^2 hybridized carbon has not been reported heretofore.[5] Although additional work is required for a detailed mechanistic proposal, our preliminary results are consistent with the premise that these reactions proceed by a methoxide-catalyzed trans addition of methanol (either concerted or stepwise) to give a tetrahedral intermediate which undergoes a methoxide induced trans elimination of hydrogen chloride (as illustrated for **2a** and **3a**).[6] In order for this mechanism to be valid, it must be assumed that nitrogen inversion is slower than rotation about the carbon–nitrogen single bond in the tetrahedral intermediate. This assumption seems reasonable since recent work indicates that nitrogen inversion is slow in *N*-benzyl-*O*,*N*-dimethylhydroxylamine.[7] It is conceivable that the lower stereoselectivity of the **3a** reaction is due to some inversion at nitrogen in the tetrahedral intermediate **7** to give its diastereoisomer **6**.[8] Inversion at nitrogen could account for the partial stereomutation of **3a** as well as the formation of **4a** during the course of the reaction.



Acknowledgments. We are indebted to the Robert A. Welch Foundation for financial support. The authors thank Dr. Timothy Rose, Texas A&M University, for making arrangements for us to use a Kahl dipolmeter.

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(4) (a) A. Werner and J. Suback, *Ber.*, **29**, 1153 (1896); (b) O. Exner, V. Jehlicka, and A. Reiser, *Collect. Czech. Chem. Commun.*, **24**, 3207 (1959); (c) R. Blaser, P. Imfeld,and O. Schindler, *Helv. Chim. Acta*, **52**, 569 (1969).

(5) All stereoselective substitution reactions at vinylic carbon proceed with retention of configuration, G. Modena, *Accounts Chem. Res.*, **4**, 73 (1971). Since submission of this work three reports on inversion during solvolysis of vinyl trifluoromethanesulfonates have appeared: (a) T. C. Clarke, O. R. Kelsey, and R. G. Bergman, *J. Amer. Chem. Soc.*, **94**, 3626 (1972); (b) T. C. Clarke and R. G. Bergman, *ibid.*, **94**, 3627 (1972); (c) R. H. Summerville and P. v. R. Schleyer, *ibid.*, **94**, 3629 (1972).

(6) The relatively small rate increase observed for the reaction of **2c** with sodium methoxide would appear to rule out an S$_N$2 type substitution mechanism.

(7) D. L. Griffith, B. L. Olson and J. D. Roberts, *J. Amer. Chem. Soc.*, **93**, 1648 (1971), and references cited therein; M. Raban and D. Kost, *J. Org. Chem.*, **37**, 499 (1972).

(8) Since **6** and **7** are diastereoisomers, it is reasonable to assume that the equilibrium between **6** and **7** lies in favor of **6**. It is not clear why **6** is more stable than **7**.

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Rate of Intersystem Crossing between ^{1}A and ^{5}A States of an Iron(II) Complex in Solution

Sir:

Octahedral complexes of transition metals possessing four to seven d electrons can exist in either high-spin or low-spin electronic configurations depending on the strength of the ligand field. An intermediate ligand field may cause the two states to possess nearly equal free energies, resulting in thermal equilibrium between the high-spin and low-spin electronic isomers of the complex.[1] The dynamics of interconversion between these states can then be investigated by suitable relaxation techniques. The results are important for understanding intersystem crossing processes in photochemically excited states and for the study of electron transfer reactions, including those in biochemical systems. We report here the observation of such a relaxation for an iron(II) complex, bis(hydrotris(pyrazolyl)borate)iron(II), $Fe(HB(pz)_3)_2$, in methanol–dichloromethane solution.

Spin-state equilibrium in $Fe(HB(pz)_3)_2$ has been established from the temperature dependence of both the magnetic moment and the pmr spectrum of the complex.[2] For the process

$$ {}^1A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} {}^5A \tag{1} $$

the following parameters have been determined in dichloromethane solution: $K = 0.47$ at 25°, $\Delta H = 3.85$ kcal mol^{-1}, and $\Delta S = 11.4$ eu.[3] We observe a comparable reversible temperature dependence of the electronic absorption spectrum. The intensity of the charge-transfer absorption between 300 and 400 nm increases sharply as the temperature is lowered. Furthermore, the shape of the absorption band is not altered significantly, confirming that the transition(s) has been correctly assigned[2] to the low-spin isomer. A least-squares analysis of the temperature dependence of the absorbance at the peak maximum at 333 nm over a temperature range from -8 to 34° yielded the parameters $K = 1.8$ at 25°, $\Delta H = 3.36$ kcal mol^{-1}, $\Delta S = 12.4$ eu, and ϵ 30,000 M^{-1} cm^{-1}. These parameters are presumably less accurate than those determined from the magnetic measurements since in the spectrophotometric case the molar absorptivity of the low-spin complex must be determined whereas the magnetic moment of the high-spin state was assumed from a determination on a related complex. The results do imply, however, that the spectral change with temperature at 333 nm can be attributed to a shift in the spin equilibrium. This permits spectrophotometric observation of the rate of the spin-state relaxation by a temperature-jump technique.

The spin-state relaxation is too rapid to observe with a conventional temperature-jump technique, and the recently developed laster Raman temperature-jump apparatus was employed.[4] This procedure requires hydroxylic solvents to absorb the Raman-shifted laser radiation at 1.41 μ, so solvent mixtures of methanol–dichloromethane were used. No significant difference was observed in the electronic absorption spectrum of the complex in the mixed solvents compared with that in pure dichloromethane. The relaxation experiments employed 1:9 and 1:1 (v/v) dichloromethane–methanol solutions. No detectable differences in relaxation times were observed for the two mixtures.

Relaxation experiments were performed with solutions of $1\text{–}6 \times 10^{-3}$ M Fe(II) at temperatures of 25 and 0°. The temperature jumps were typically about 1° and the associated absorbance decreases were of the magnitude calculated from the "static" temperature dependent spectrum. The relaxation was first order with a lifetime, averaged from a number of experiments, of 32 ± 10 nsec. Within the rather large experimental error occasioned by the low signal-to-noise ratio, the relaxation time is independent of temperature and of concentration over the range studied. Repeated temperature jumps of the same sample did not result in irreversible decomposition of the solution as determined by its electronic absorption spectrum.

We identify the observed relaxation process with the reestablishment of the perturbed equilibrium of reaction 1. The k_1 and k_{-1} values calculated from the relaxation time and the equilibrium constant for the reaction are 1×10^7 and 2×10^7 sec^{-1}, respectively, at 25°. These rate constants appear to be reasonable for an intersystem crossing process for which $\Delta S = 2$, and a fairly substantial coordination sphere reorganization is expected.[5] Although the transition involves two spin changes, it can take place on a single potential energy surface since, as a consequence of spin–orbit coupling, the two states have an irreducible representation in common (A_1 in the D_{3d} double group).[6]

The activation enthalpies consistent with the small temperature dependence of the rate constants are $\Delta H_1{}^{\ddagger} \leq 4$ kcal mol^{-1} and $\Delta H_{-1}{}^{\ddagger} \leq 0$. The similarity of $\Delta H_1{}^{\ddagger}$ and ΔH implies in the simplest, but not necessarily the only, model that the activated complex resembles the products and thus that $\Delta S_1{}^{\ddagger} \approx \Delta S - R \ln 5 = 8.2$ eu.[3,7] The value of the transmission coefficient calculated on this basis from the expression

$$ k = \kappa 10^{13} e^{-\Delta H^{\ddagger}/RT} e^{\Delta S^{\ddagger}/R} \tag{2} $$

is about 10^{-5}. This estimate of κ must be regarded as tentative at this time.[8]

If the relaxation observed in this system is typical of other metal complexes, it suggests that a change in spin state is probably not rate determining in most electron transfer reactions studied in solution.[9] It might be possible to establish conditions using high concentrations of reactants in which a nearly diffusion-controlled electron transfer reaction proceeds more rapidly than the spin-equilibration process. However, provided the coordination sphere reorganizations are

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(1) R. L. Martin and A. H. White, *Transition Metal Chem.*, **5**, 113 (1969).

(2) J. P. Jesson, S. Trofimenko, and D. R. Eaton, *J. Amer. Chem. Soc.*, **89**, 3158 (1967).

(3) The entropy term contains a contribution of $R \ln 5 = 3.2$ eu from the spin degeneracy of the quintet state.

(4) J. V. Beitz, G. W. Flynn, D. H. Turner, and N. Sutin, *J. Amer. Chem. Soc.*, **92**, 4130 (1970); D. H. Turner, G. W. Flynn, N. Sutin, and J. V. Beitz, *J. Amer. Chem. Soc.*, **94**, 1554 (1972).

(5) The Fe–N bond lengths will be different in the high-spin and low-spin states.

(6) The double group, which corresponds to the direct product of the coordinate and spin space, is the one to use for the case of significant spin–orbit coupling; see G. Herzberg, "Electronic Spectra of Polyatomic Molecules," Van Nostrand, Princeton, N. J., 1967.

(7) Although values of $\Delta H_1{}^{\ddagger} < \Delta H$ are, of course, also consistent with the temperature dependence of the rates, such an assignment would require a negative value of $\Delta H_{-1}{}^{\ddagger}$.

(8) An alternative interpretation is that $\kappa \approx 1$ and that the activated complex is much more restricted than the reactants in its motions ($\Delta S_1{}^{\ddagger} = -15$, $\Delta S_{-1}{}^{\ddagger} = -29$ eu); such a restriction in the motions of the activated complex is unlikely in view of the low activation enthalpies and the simplicity of the reaction and for these reasons the first interpretation ($\kappa \approx 10^{-5}$) is preferred.

(9) In preliminary experiments we observed a half-life of ≤ 15 nsec for relaxation of the spin-state equilibrium in $Co(terpy)_2{}^{2+}$ in aqueous solution, for which $\Delta S = 1$ and a smaller coordination sphere reorganization is expected.

small, it is likely that equilibrium between the spin states will be maintained under the usual experimental conditions.

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Studies on the Prostaglandin A_2 Synthetase Complex from *Plexaura homomalla*

Sir:

Recent research has established that the gorgonian *Plexaura homomalla* (*ex* Caribbean) is an unusually rich source (1.8% net weight) of prostaglandin A_2 (PGA_2) and its acetoxy methyl ester.[1-3] We were intrigued by the possibility that the prostaglandin synthetase of this easily available "sea whip" variety of coral might be highly active and/or abundant and, further, by the chance that the enzyme(s) might be solubilized, purified, and affixed to a column so as to allow an *in vitro* enzymic synthesis of prostaglandins in a continuous flow system. In addition it seemed that the study of the *Plexaura homomalla* synthetase complex might lead to worthwhile insights regarding the biosynthesis of prostaglandins. The following summarizes our endeavors to isolate and characterize this enzyme complex.

A small sample of *Plexaura homomalla* was collected *ca.* 0.5 mile north of McArthur's point, New Providence, Bahamas, and was immediately packed in Dry Ice to preserve enzymatic activity.[4] Examination of the indigenous prostaglandin isolated by extractions with ethyl acetate confirmed that the *Plexaura homomalla* in this area contain exclusively prostaglandins with the 15*S* (natural mammalian) configuration.[2]

We were disappointed to find that the incubation of tritiated arachidonic acid with a freshly prepared homogenate of *P. homomalla* in 0.05 *M* Tris or phosphate buffer at pH values from 6 to 8.5 and 28–29° did not result in the production of significant amounts of PGA_2, its methyl ester, or 15-*O*-acetyl derivatives. However, it was found that if the buffered homogenate were prepared using sea water, the enzymic synthesis of PGA_2 could be detected easily. It was further determined that the activity or lack of same of the PGA_2 synthetase depended simply on the presence of an appropriate concentration of sodium chloride.

Utilizing our observations that this prostaglandin synthetase has an optimum pH range of 7.5–8.0 and requires an optimum salt concentration of 1 *M*, we developed the following procedure to obtain active preparations of soluble PGA_2 synthetase. Frozen coral (*ca.* 2 g) was cut into small pieces and placed into a blender cup with 20 ml of ice-cold pH 8.0 buffer (0.05 *M* Tris and 1 *M* NaCl). The Waring Blendor was operated for 2.5 min on 70 V, and the resulting homogenate was centrifuged for 20 min at 8000*g*. The supernatant fraction was centrifuged at 100,000*g* for 90 min. The microsomal pellet contained the prostaglandin synthetase. Solubilization was effected by sonication of the pellet for 45 sec in 10 ml of pH 8.0 buffer (0.05 *M* Tris, 1 *M* NaCl) (0°) and the nadding sufficient glycerol and Triton X-100 to bring the final concentration of the latter to 5 and 0.5%, respectively.[5] This treatment did not enhance activity and did not stabilize the synthetase complex, since 80% of the activity was lost after standing 7 hr at 0°.

The incubation procedure was as follows. One milliliter of the above solution diluted 1:1 with buffer (pH 8.0, 0.05 *M* Tris, 1 *M* NaCl) was incubated with 25 μg of 2-tritioarachidonic acid for 20 min at 29°. After addition of 1 g of NaH_2PO_4 to quench the reaction, the solution was extracted five times with ethyl acetate. The residue, after removal of the solvent, was chromatographed on 20-cm long Brinkmann F-254 silica gel tlc plates using two developments with 2% acetic acid, 7% dioxane, and 91% benzene. Three major product bands were found: band I (10%) with an R_f of 0.4 was PGA_2; bands II (40%) and III (20%) (the identity of which are unknown) moved with R_f values of 0.52 and 0.62, respectively.[6]

Band I was confirmed to be PGA_2 by converting the extracted band to PGA_2 methyl ester using diazomethane and then to the 15-trimethylsilyl ether of PGA_2 methyl ester. In each case the labeled product displayed chromatographic behavior identical with the standard. Final confirmation was obtained by preparing the semicarbazone of band I, mixing it with the semicarbazone of authentic PGA_2,[7] and recrystallizing the mixture. Constant activity and constant melting point were obtained

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(1) A. J. Weinheimer and R. L. Spraggins, *Tetrahedron Lett.*, 5185 (1969).

(2) (a) W. P. Schneider, R. D. Hamilton, and L. E. Rhuland, *J. Amer. Chem. Soc.*, **94**, 2122 (1972); (b) F. Alvarez, D. Wren, A. Prince, and J. Fried (Syntex Corporation) kindly informed us of their unpublished discovery of A prostaglandins having the natural 15*S* configuration in *P. homomalla* from certain Caribbean areas (private communication, Nov 1971).

(3) R. J. Light and B. Samuelsson, *Eur. J. Biochem.*, **28**, 232 (1972).

(4) If the coral is allowed to stand for 6 hr at 0°, the activity of the prostaglandin synthetase preparation diminishes by 60%.

(5) Solubilization was established, since the synthetase could not be precipitated by centrifuging at 100,000*g* for 30 min.

(6) Bands II and III do not seem to be derived from the postaglandin synthetase. The enzymes responsible for their production can be separated by either $(NH_4)_2SO_4$ precipitation or by column chromatography on agarose.

(7) We thank Drs. John Fried and Otto Halpern of Syntex Research for generous samples of PGA_2.