for the 2D experiment. The separation of the CSA powder patterns in a 2D spectrum removes the overlap that is often severe in the one-dimensional spectrum of a static sample, revealing the useful CSA information.

## 17-*O*-Acetyltestosterone Formation from Progesterone in Microsomes from Pig Testes: Evidence for the Baeyer-Villiger Rearrangement in Androgen Formation Catalyzed by CYP17

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Androgens are formed by testicular cytochrome P450 CYP17[1] via an initial 17$\alpha$-hydroxylation of progesterone (P) or pregnenolone followed by the cleavage of the C-17 side chain.[2] Although the cleavage reaction observed in testes has been shown to utilize NADPH and molecular oxygen,[3] a definitive mechanism for the reaction has evaded investigators. In this communication we report the NADPH-dependent formation of 17-*O*-acetyltestosterone (AT) upon incubation of P with microsomes and purified CYP17 from perinatal pig testes. The formation of AT suggests that this enzyme has the capacity to catalyze the Baeyer–Villiger rearrangement via a ferric heme peroxy substrate intermediate.

The formation of AT, 17$\alpha$-hydroxyprogesterone (17$\alpha$-OHP), and androstenedione (A) was analyzed by HPLC with a radio-flow detector. The products were eluted from a 5-$\mu$m ODS column isocratically with 30% methanol, 25% acetonitrile, and 45% water at a flow rate of 1 mL/min.[4] AT was identified by comigration with authentic standard (retention time 73 min) and confirmed by GCMS. Under electron impact ionization (70 eV; source temperature 150 °C), the parent compound had a molecular weight of $m/z$ 330 with a major fragment corresponding to the loss of acetate ($M^+/-60$). No ion corresponding to the loss of water ($M^+/-18$) was observed.

In microsomes the formation rate of AT was 1 pmol/min/mg of protein as compared to 305 and 45 pmol/min/mg for 17$\alpha$-OHP and A, respectively, while the activity associated with the purified enzyme was 1.6, 447, and 326 pmol/min/nmol of P450 for AT, 17$\alpha$-OHP, and A, respectively, under the conditions described in ref 4. AT formation was NADPH-dependent in both systems.

If AT is formed by CYP17, then it and 17$\alpha$-OHP should arise from the same CYP17–progesterone complex and their rates of formation should be equally sensitive to competitive inhibitors. Ketoconazole, a well-characterized competitive inhibitor of CYP17,[5] and pregnenolone, the cosubstrate for the reaction, did not differentially inhibit the formation of either product. The $IC_{50}$ values, associated with ketoconazole inhibition were 1.60 ± 0.74 and 2.27 ± 0.20 $\mu$M, respectively, for AT and 17$\alpha$-OHP (Figure 1),[6] and the apparent $K_i$ values associated with pregnenolone inhibition (determined by the method of Dixon) were 5.0 ± 3.5 $\mu$M for AT and 9.6 ± 4.6 $\mu$M for 17$\alpha$-OHP. The formation of both products by purified CYP17 was inhibited 48 and 51% by 5 $\mu$M ketoconazole, respectively. These data strongly suggest that AT is formed from the same enzyme–substrate complex as 17$\alpha$-OHP.

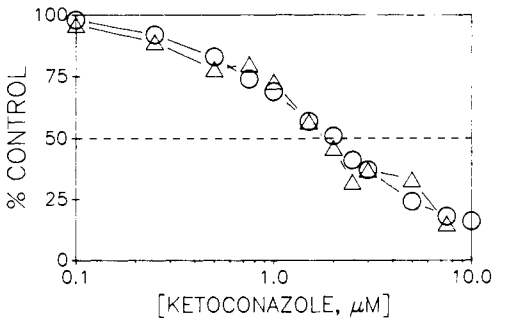


**Figure 1.** Inhibition of CYP17 activities in microsomes from pig testes by ketoconazole. The effect of ketoconazole upon the formation of AT (△) and 17$\alpha$-OHP (○) was determined in 10-min incubations at 37 °C containing 1.5 mg/mL of protein and 50 $\mu$M [$^3$H]progesterone.

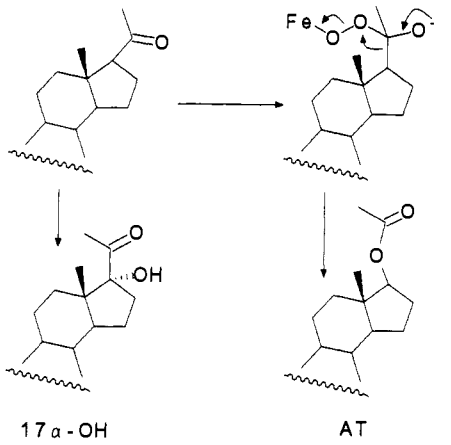


**Figure 2.** Proposed mechanism in the formation of 17-*O*-acetyltestosterone via the Baeyer–Villiger rearrangement.

The insertion of an oxygen in a carbon–carbon bond is most readily accomplished via rearrangement of a peroxy intermediate. This well-characterized chemical reaction is known as the Baeyer–Villiger rearrangement.[7] The rearrangement of the C-20 ferric peroxide of progesterone via the Baeyer–Villiger rearrangement would result in the formation of AT (Figure 2). Precedent for this type of reaction being catalyzed by a cytochrome P450 was recently shown in a report by Fisher and co-workers.[8] They reported evidence that lanosterol 14$\alpha$-demethylase catalyzes the formation of 14$\alpha$-(formyloxy)lanost-8-en-3$\beta$-ol from lanosterol and concluded that this could only arise via the Baeyer–Villiger rearrangement.

Since AT has not yet been shown to be an intermediate in A formation from P, it does not directly implicate this mechanism in androgen formation by CYP17. It is possible that this is a process that occurs infrequently (leakage). Reports in the literature, primarily by Akhtar and co-workers,[9] propose the in-

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(4) The microsomal incubations typically contained 1.5 mg/mL of microsomal protein from pig testes, 25 $\mu$M [$^3$H]progesterone, 50 mM potassium phosphate (pH 7.25), 3 mM magnesium chloride, and 1 mM NADPH. The incubations with the reconstituted system contained 0.22 $\mu$M CYP17, 5000 units/mL cytochrome P450 reductase, and 5 $\mu$g/mL dilauroylphosphatidylcholine. CYP17, purified by the method of Suhara (Suhara, K.; Yoshiyuki, F.; Shiroi, M.; Katagiri, M. *J. Biol. Chem.* **1984**, *256*, 8729–8736), produced a single band by SDS gel electrophoresis. Cytochrome P450 reductase was purified by the methods of Dignam and Strobel (Dignam, J. D.; Strobel, H. W. *Biochem. Biophys. Res. Commun.* **1975**, *63*, 845–852) and Yasukochi and Master (Yasukochi, Y.; Masters, B. S. S. *J. Biol. Chem.* **1976**, *251*, 5337–5344).

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0002-7863/92/1514-8309$03.00/0 

volvement of a peroxy intermediate in C-17 side-chain cleavage; however, rearrangement to androgen via the Baeyer-Villiger reaction was not considered an option. We feel that, in light of the above finding, this rearrangement must be considered a viable option. It is consistent with the studies of Akhtar in which $^{18}O$ was incorporated into acetate and is also consistent with the formation of the $\Delta^{16}$ steroids, which are formed by loss of the 17$\beta$ side chain and the 16$\alpha$-hydrogen.[9]

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## Salt-Induced, Ligand-Controlled, Intra- vs Intermolecular Electron Transfer in a Fulvalene-Bridged Organoiron Diradical

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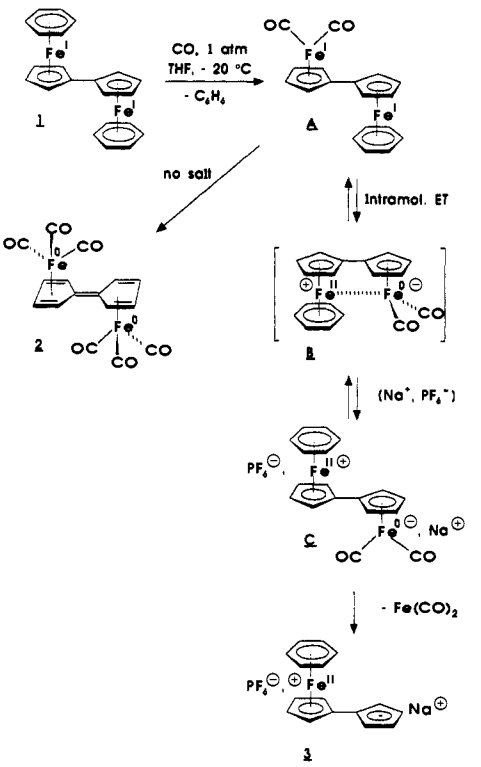
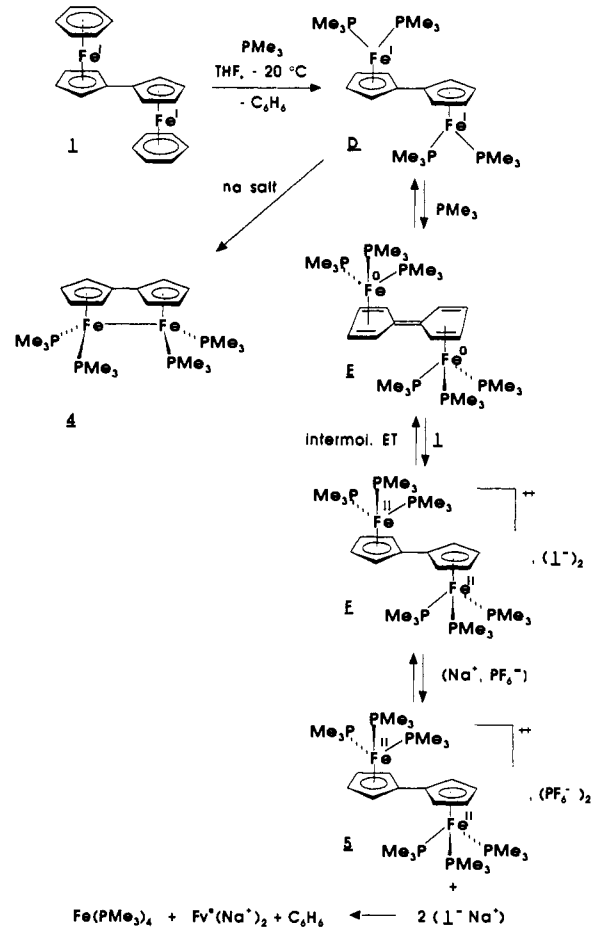
We report that a $Na^+$ salt induces intramolecular electron transfer (ET) between the two iron redox centers of the fulvalene-bridged diradical **1** upon ligand exchange and that this ET can be switched to intermolecular ET by controlling the donicity of the incoming ligand. This special salt effect[1] is shown here to bear a synthetic potential in transition metal chemistry due to the facility of these elements to change their redox states.[2-4]

The reaction of the bis 19-electron complex $[Fe^I_2(\mu_2,\eta^{10}\text{-Fv})(\eta^6\text{-}C_6H_6)_2]$ (**1**)[5] (Fv = fulvalene)[6] at -20 °C with 1 atm of CO in THF in the absence of $Na^+PF_6^-$ leads as expected to the replacement of both benzene ligands by six CO's, giving the slightly unstable new red diamagnetic complex **2**[7] which indicates elec-

**Scheme I**




**Scheme II**

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