ion pairs (eq 6) do not differ significantly from those reported for complete ionization ($K = 10^{-3}$ to 10^{-16}),[9] then the observed rate of exchange is too rapid for this type of exchange mechanism. Similar arguments tend to preclude

$$CH_3HgX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CH_3Hg^+ \,||\, X^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} CH_3Hg^+ + X^- \qquad (6)$$

exchange involving one solvent separated ion pair (eq 3) even if a fast second-order diffusion controlled exchange ($k_2 = 10^9$ to 10^{10}) were assumed

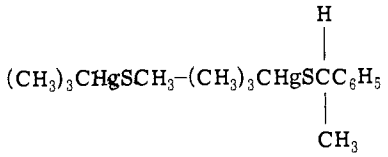
$$\text{rate} = \frac{k_2}{2} K_1[CH_3HgX][CH_3HgCN]$$

However, the ionic mechanism in eq 2

$$\text{rate} = \frac{k_2}{2}(K_1[CH_3HgX])^{1/2}[CH_3HgCN]$$

is not as readily excluded with more ionic substrates. Likewise it is plausible from Simpson's work[1] that at higher acetate ion concentrations the CH_3HgCN-CH_3HgOAc system may undergo exchange by the anionic mechanism in eq 1.

The trend observed with the rate constants also establishes the importance of bridging in the transition state. Thus, the slowest rate observed is for **2a** (Table I) where a chlorine bridge is presumably involved. The faster rate of exchange for the *tert*-butyl mercaptide (**2h**) relative to the methyl mercaptide (**2e**) suggests that electron density at sulfur is more important than steric effects. The apparent importance of bridging in the above reactions suggested that a RHgSR-RHgSR′ exchange would exhibit a rate of exchange sufficiently high that an ionic mechanism could be unequivocally excluded. Our anticipations were realized with the exchange system



These compounds exhibited a line separation for the *tert*-butyl resonance of 13.5-14.5 Hz and were sufficiently soluble in the mixed solvents HCF_2Cl:$HCFCl_2$ (4:1) at a coalescence temperature of −142° to allow a total line shape analysis exchange study. The free energy of activation for this remarkably facile anion exchange was found to be only 5.2 kcal/mol at −138°. Extrapolation of the data obtained at low temperature with several different ratios of substrate to 25° gave $\Delta G^{\ddagger}_{25} = 7.3$ kcal/mol and $\Delta S^{\ddagger} = -12.7$ eu. This exchange reaction also exhibited second-order kinetics with extrapolated $k_2 = 3 \times 10^7$ at 25° calculated on the basis of exchange as in eq 5. Since the equilibrium constant for ionization of a typical RHgSR is $\sim 10^{-16}$ in a polar solvent, any calculated rate constants based upon the ionic processes eq 1-4 for the relatively nonpolar solvent system investigated would be at least 10^4 greater than that of a diffusion controlled process. These data thus provide the first unequivocal example of a bimolecular anion exchange of RHgX with total exclusion of an ionic process. In conclusion, our results provide convincing evidence that all anion exchange reactions of RHgX compounds involving a covalent ligand bonded to mercury proceed via a bimolecular process involving a bridged intermediate such as **1**.[10]

Acknowledgment is made to the National Institutes of Health (ES-00761-03) for support of this work.

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$$\ln\frac{k}{T} = \ln\frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} - \left(\frac{\Delta H^{\ddagger}}{R}\right)\left(\frac{1}{T}\right)$$
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(9) The K_{eq} for the reaction of $CH_3Hg^+ + X^- \rightleftarrows CH_3HgX$ for $X^- = OAc^-$, Cl^-, SCN^-, Br^-, CN^-, $C_6H_5S^-$, and $HOCH_2CH_2S^-$ are $10^{3.6}$, $10^{5.25}$, $10^{6.05}$, $10^{6.62}$, $10^{14.1}$, $10^{14.67}$, and $10^{16.1}$, respectively, in aqueous solution. Equilibrium constants (K_1) for formation of solvent separated ion pairs are not available. However, it is intuitively reasonable that the same relative order of ionic character should be maintained.
(10) Extended Huckel calculations suggest that the bridged transition state 1 is considerably more stable than an acyclic species. The calculations also provide convincing evidence for significant Hg–Hg bonding in the transition state (unpublished results).

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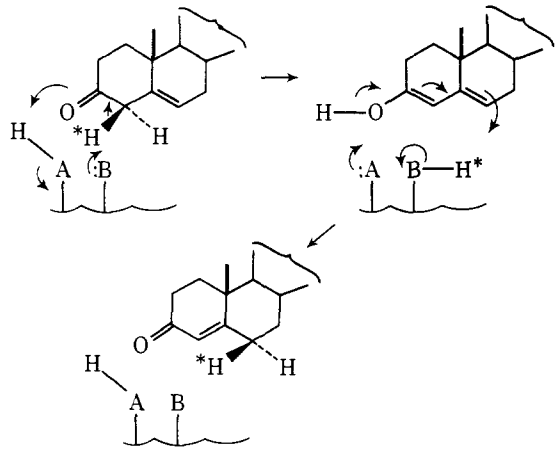
Irreversible Inhibition of Δ^5-3-Ketosteroid Isomerase by 5,10-Secosteroids

Sir:

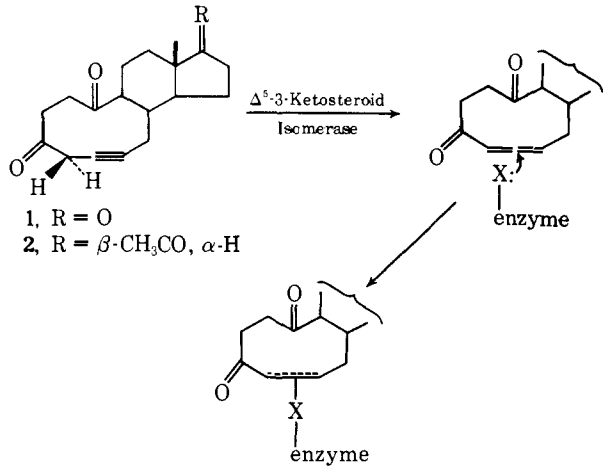
Recent studies[1] have shown that remarkably specific irreversible enzyme inhibitors can result from compounds bearing potential reactive groupings which are unmasked at the active site by the target enzyme. This specificity resides in the generation of the alkylating agent by the target enzyme at the active site as a result of the enzyme's normal catalytic process. The process is exemplified by the enzymatic conversion of an acetylenic compound to an allene which can alkylate an active site amino acid residue. The first such example was provided by Bloch[2] who showed that the acetylenic analog of a normal substrate for β-hydroxydecanoyl thioester dehydrase is converted by the enzyme to the corresponding conjugated allenic thioester with rapid alkylation of an active site histidine residue. This approach has been applied to the inhibition of monoamine oxidase[3] and γ-cystathionase.[4]

The enzyme Δ^5-3-ketosteroid isomerase[5] (EC 5.3.3.1) from *Pseudomonas testosteroni* converts C_{19} and C_{21} Δ^5-3-ketosteroids to the corresponding Δ^4-3-ketosteroids. The proposed mechanism[5,6] involves removal of the axial 4β-hydrogen with concomitant enolization to give a $\Delta^{3,5}$-dienol, followed by ketonization with axial reprotonation at C-6. The hydrogen transfer from C-4 to C-6 is intramolecular (Scheme I). This reaction when carried out by mamma-

Scheme I



Scheme II



lian Δ^5-3-ketosteroid isomerases is a key step in the biosynthesis of steroid hormones.

We report here the rapid irreversible inhibition of bacterial Δ^5-3-ketosteroid isomerase by acetylenic steroid analogs (illustrated by **1** and **2**) (Scheme II). A plausible mechanism involves conversion of the β,γ-acetylenic ketone to the conjugated allenic ketone via enzymatic enolization followed by ketonization at C-3 with protonation at C-6. The conjugated allenic ketone should then react readily with a nucleophilic residue at or near the active site. This process finds analogy in studies with β-hydroxydecanoyl thioester dehydrase.[2]

Compounds **1**[7] and **2**[8] were synthesized by closely similar routes. Thus, 3β,17β-diacetoxyestr-5(10)-en-6-one (**3**) was prepared by direct chromium trioxide–pyridine oxidation[9] of 3β,17β-diacetoxyandrost-5-en-19-ol. Epoxidation of **3** with *m*-chloroperbenzoic acid in benzene (reflux) or by reaction with alkaline hydrogen peroxide followed by reacetylation gave the corresponding 5β-10β-oxidosteroid (**4**), mp 245–247°. Fragmentation of **4** with *p*-toluene sulfonylhydrazine[10] at ambient temperature in acetic acid–chloroform (1:1) gave 3β,17β-diacetoxy-5,10-secoestr-5-yn-10-one (**5**), mp 204–206°, in high yield. Hydrolysis of the acetate groups in **5** (3% methanolic KOH) followed by oxidation with Jones reagent[11] gave **1**. Compound **2** was synthesized by exactly the same sequence of reactions, starting with 3β,20-diacetoxy-5-pregnen-19-ol.

Incubation[12] of crystalline Δ^5-ketosteroid isomerase at pH 7.0 with acetylenic steroids **1** and **2** in 1,4-dioxane resulted in rapid irreversible and complete inactivation of the enzyme. The inactivation was progressive with time, and

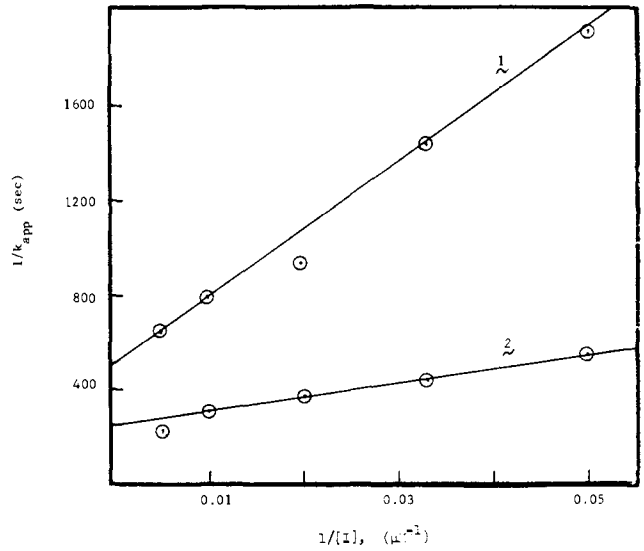


Figure 1. Irreversible inactivation of Δ^5-3-ketosteroid isomerase of *P. testosteroni* by 5,10-secoestr-5-yne-3,10,17-trione (**1**) or 5,10-seco-19-norpregn-5-yne-3,10,20-trione (**2**). Double reciprocal plots are shown of the pseudo-first-order rate constant of inactivation (k_{app}) with respect to inhibitor concentrations. The values of K_I and k_3 for **1** and **2** were determined from the slopes and intercepts. The regression lines were drawn according to a least-squares fit.

half-lives in the range of 150–1320 sec were observed at concentrations of 20–200 μM of the two inhibitors. The enzymatic activities of control vessels which received only equivalent volumes of 1,4-dioxane remained constant at initial activities. Evidence for the irreversible nature of the inhibition is based on: inability to restore enzymatic activity by prolonged dialysis (24 hr at 4° vs. 1 mM potassium phosphate buffer, pH 7.0); the fact that extensively diluted partially inhibited enzyme preparations retained constant activity for many days; and the kinetic behavior described below.

The initial rates of inactivation of the isomerase by **1** and **2** could be analyzed by the method of Kitz and Wilson[14] since very satisfactory pseudo-first-order behavior was observed. It is assumed that [I] (inhibitor) $\gg$ [E] (enzyme), that [E·I] (the reversible enzyme–inhibitor complex) is at all times in equilibrium with enzyme and inhibitor, and that $k_{cat} \gg k_{inh}$. The scheme for the formation of EI′ (the irreversible enzyme–inhibitor derivative) may then be represented as follows.

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E \cdot I \xrightarrow{k_3} EI'$$

If the enzyme–inhibitor solution is diluted extensively prior to assay, the active enzyme (ϵ) = [E] + [E·I]. Then $-d(\epsilon)/dt = k_3[E \cdot I]$, and $K_I = ([E][I])/[E \cdot I]$. Thus

$$\ln \frac{(\epsilon)}{[E_t]} = \frac{-k_3}{1 + (K_I/[I])}$$

where E_t = total amount of enzyme in system.

If we define

$$k_{app} = \frac{-k_3}{1 + (K_I/[I])}$$

then

$$\frac{1}{k_{app}} = \frac{1}{k_3} + \frac{K_I}{k_3[I]}$$

Consequently double reciprocal plots of k_{app} with respect to [I] should be linear, with slopes and intercepts permitting the determination of K_I and k_3 where the latter is the over-

all rate constant for the irreversible inhibition process.

From plots of ln ϵ (residual enzymatic activity) vs. time, k_{app} was determined at five inhibitor concentrations for both **1** and **2.** Strict linearity of the semilogarithmic plots was observed in all cases, over greater than two half-lives. A plot of $1/k_{app}$ vs. $1/[I]$ was linear and gave k_3 and K_I (Figure 1). For compound **1,** $K_I = 56\ \mu M$ and $k_3 = 1.98 \times 10^{-3}$ sec^{-1}, and for compound **2,** $K_I = 32\ \mu M$ and $k_3 = 4.10 \times 10^{-3}$ sec^{-1}.

These experiments suggest that the acetylenic steroid analogs **1** and **2** inactivate Δ^5-3-ketosteroid isomerase by covalent linkage to the enzyme. The inactivation is rapid and specific, presumably because the isomerase enzyme generates the alkylating system at its active site by exercising its normal catalytic function.

In conclusion, we have shown for the first time that Δ^5-3-ketosteroid isomerase can be inhibited irreversibly and very efficiently by compounds designed to act in such a manner. The β,γ-acetylenic ketosteroid analogs described here are of special interest, not only as tools for further study of the precise mode of action of isomerase but also as potential inhibitors of steroid hormone biosynthesis. Both these matters are under active investigation in these laboratories.

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Infrared Laser Induced Reaction of CF_2Cl_2

Sir:

There have been a number of reports of chemical reactions in gases induced by infrared lasers,[1] where the laser frequency is in resonance or near-resonance with a vibrational frequency of a reacting molecular species. If the laser energy fed into that vibrational mode is dissipated too rapidly into other vibrational and translational modes of the system, the net effect will be one of simple heating. Temperatures easily exceeding 1000° may be produced[2] at the laser beam power densities and the optical absorption densities of the gases used in most of the reported experiments.

Such high temperatures and the extreme temperature gradients surrounding the laser beam are difficult to simulate using purely thermal (i.e., nonlaser) techniques. Thermodynamically, the conditions are quite different from those usually employed in thermal reaction studies. Therefore, the fact that a laser produces a reaction different from that observed "thermally" should not be considered as confirming evidence that the reaction is "bond selective", with energy confined to a selected vibrational mode or molecular species.

This note reports on the reaction of CF_2Cl_2 (Freon 12) induced with a tunable CO_2 laser and particularly on some experiments designed to show that the reaction is not due to simple heating.

We find that laser frequencies in the range 929–935 cm^{-1} convert CF_2Cl_2 into $C_2F_4Cl_2$ (Freon 114) and Cl_2. Gas chromatograph and mass spectrometer analyses show no evidence of other products. Beam powers from 0.5 to 5.5 W were used, with a long focal length mirror producing a 2 mm diameter beam that passed through a gas cell 1 in. diameter by 4 in. long. Initial pressures of CF_2Cl_2 were chosen from 50 to several hundred Torr. The sampling beam of an infrared monochromator[3] was passed through the cell transverse to the laser beam, and by monitoring the absorbtion of $C_2F_4Cl_2$ at 1050 cm^{-1}, the rate of product formation during irradiation was measured. The same monochromator was used to measure the laser frequency prior to each run.

The reaction saturates in time and does not go to completion. In all cases, the data show the concentration N of the Freon 114 product growing with time t according to the relation $N = N_s[1 - \exp(-\lambda t)]$, where the saturation concentration N_s and the effective rate coefficient λ depend on laser intensity and frequency and on the starting Freon 12 pressure. (Details will be given in a later publication.) Since the Freon 114 product also absorbs strongly in the 929–935-cm^{-1} range of exciting frequencies, it would appear that saturation results from a reverse reaction driven by the laser. The reverse reaction, however, is not found to occur in a starting mixture of Freon 114 and Cl_2. This suggests that the reverse reaction occurs between Freon 114 and atomic chlorine produced during irradiation of Freon 12. The presence of Cl in this state is indicated by its rapid reaction with other gases, such as NH_3, that may be introduced.

To show that the reaction of CF_2Cl_2 is not produced by simple heating, a mixture of 100 Torr of CF_2Cl_2 and 4.4 Torr of SF_6 was irradiated (a) first with 5 W of laser power at 949 cm^{-1}, where the optical absorbtion coefficient of SF_6 is 4.3 cm^{-1}, while that of CF_2Cl_2 is negligible, and (b) then with 5 W at 935 cm^{-1}, where the optical absorbtion coefficient of CF_2Cl_2 is 4.3 cm^{-1}, while that of SF_6 is negligible. The pressures of the two gases are chosen to give identical absorbance at their respective frequencies, so that the laser power absorbed per unit volume should be the same in each case. Accordingly, simple conversion into heat would produce similar temperature distributions. (Actually, conditions are not quite identical because the absorbtion coeffi-