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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(8) 5,10-Seco-19-norpregn-5-yne-3,10,20-trione (**2**): mp 158–159°; mass spectrum *m/e* 314 (M^+), 286, 271.
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(12) Crystalline Δ^5-3-ketosteroid isomerase[13] was used in all studies and dialyzed immediately prior to use. The inactivation experiments were carried out at 26.5° in a final volume of 500 μl. For experiments with compound **1** the reaction vessel contained: 7.22 μM isomerase enzyme [based on a subunit weight of 13,394 daltons[5]], 1.0 mM potassium phosphate buffer (pH 7.0), and **1** (varied over a 20 to 200 μM range) in 1,4-dioxane (20 μl). Experiments with compound **2** were identical with **1**. Aliquots were removed at 1- or 2-min intervals, diluted (as much as 1.5×10^6 fold in 1% neutral bovine serum albumin), and assayed for residual enzymatic activity (ϵ) in the presence of 57.8 μM Δ^5-androstene-3,17-dione ($Km = 340\ \mu M$)[5] by monitoring the appearance of the conjugated ketone chromophore at 248 nm in water.
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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-

cients are measured at low intensity while optical transparency will be induced at high intensity; the latter effect, however, is comparable for the two gases used here.)

We fine *no* reaction in case (a) above, while (b) proceeds at nearly the same rate and with the same products as if SF_6 were absent. In fact, a null result is obtained at the 949-cm^{-1} frequency with SF_6 pressures through 20 Torr, or with laser power increased to 8 W. By comparison, at 935 cm^{-1}, we find the reaction rate grows as the eleventh power of laser intensity.

In other experiments, various amounts of helium were added to 100 Torr of CF_2Cl_2. The reaction rate was virtually uneffected for He pressures up to 40 Torr. Since the latter addition increases the macroscopic thermal conductivity by a factor of 5, one expects a significant decrease in the temperatures produced. Because a thermal reaction rate would vary exponentially with reciprocal temperature, the experimental result may be considered as further evidence against a single heating mechanism.

The vibrational mode of CF_2Cl_2 excited in these experiments is believed to represent a rocking motion of the CF_2 group against the Cl_2 group[4,5] (or vice-versa). We note also that under isothermal conditions, CF_2Cl_2 is reported to be entirely stable below 700°, decomposing entirely at 900° to products other than those found here,[6] while Freon 114 decomposes entirely[7] at 500°.

Freon 12 has been studied[8] as a saturable absorber for laser mode locking. At the pressures used (~1 Torr) the reaction rate is too small for convenient measurement, but it may be large enough in the long run to prevent the application mentioned.

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On the Fluorescence of Bilirubin[1]

Sir:

Recently we reported on the low temperature fluorescence of biliverdin and biliverdin dimethyl ester,[2] both of which exhibited a two-fluorescence band system with emission maxima at 725 and 480 nm. At that time the fluorescence of *free* bilirubin (**1**) was difficult or impossible for us to detect. Indeed, whether free bilirubin fluoresces or whether the fluorescence can even be detected has been the subject of controversy;[3] whereas, it has been clearly established that bilirubin fluoresces when it is *bound* to human serum albumin,[3,4] rabbit, horse, porcine, or sheep albumin.[3] In order to study the fluorescence of free bilirubin, we resorted to the use of micelle forming detergents in aqueous solution[5] and in EPA solvent. We report herein the first observation of free bilirubin fluorescence in a micellar environment at room temperature and at 77°K.

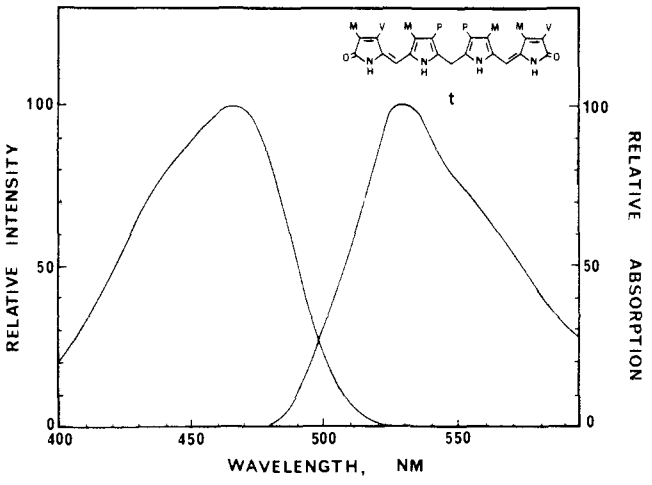

Figure 1. Room temperature fluorescence and excitation spectra of bilirubin (**1**) (5.2 mg) in 100 ml of water containing 20.5 mg of cetyltrimethylammonium bromide. Excitation wavelength and bandpass were 440 and 10 nm, respectively: M = CH_3, V = $CH{=}CH_2$, and P = CH_2CH_2COOH.

Bilirubin (**1**)[6] is insoluble in water and common organic solvents, although it exhibits very limited solubility in a few organic solvents, e.g., chloroform, benzene, and dimethyl sulfoxide. However, it is readily solubilized in water and some organic solvents in the presence of a detergent such as cetyltrimethyl ammonium bromide (CTAB).[7a] Spectroquality EPA[7b] (ether–isopentane–ethanol, 5:5:2) and glass distilled water were used as solvents in this work. In distilled water containing 5.2 mg % **1** and 20.5 mg % CTAB we observed room temperature fluorescence emission[8] at 530 nm from **1** when the excitation wavelength used was 440 nm[9] (Figure 1). When the excitation wavelength used was the more typical 390 nm, only an extremely low level of and barely detectable fluorescence emission was observed. These findings support the disputed[3] observation of Beaven et al.[4] of an extremely weak fluorescence from **1** in aqueous solution at pH 8.4. The fluorescence of **1** at room temperature, in neutral solution with added detergent and without albumin, is clearly established.

The fluorescence of bilirubin in nonaqueous solvents has not been published heretofore, although observations of bilirubin fluorescence (λ 525 nm) have been made for a methanol (with added ammonia) glass solution.[10] We have found fluorescence emission[11] from **1** in EPA glass at 77°K with and without added detergent (CATB)[9]: in 10^{-5} to 10^{-6} *M* solutions of **1** alone and in the presence of monomeric (10^{-4} to 10^{-5} *M*) CATB as well as excess (10^{-3} *M*) CATB. By varying the concentration of CATB, in EPA the fluorescence λ_{max} shifts from 530 to 505 nm from pure EPA to 46.4 mg % CATB in EPA (Figure 2), respectively. Shore and Turro[12] have used this type of spectral shift to deduce the critical micelle concentration (cmc) of a host detergent from the inflection point in the fluorescence vs. detergent concentration plot. They determined a cmc $\simeq 8.8 \times 10^{-4}$ *M* for cetyltrimethylammonium bromide using 11-[3-hexyl-1-indolyl]-undecyltrimethylammonium bromide as a fluorescent probe. Similarly, by using **1** as a fluorescent probe, we have determined the CATB cmc to be $\sim 2 \times 10^{-4}$ *M*.