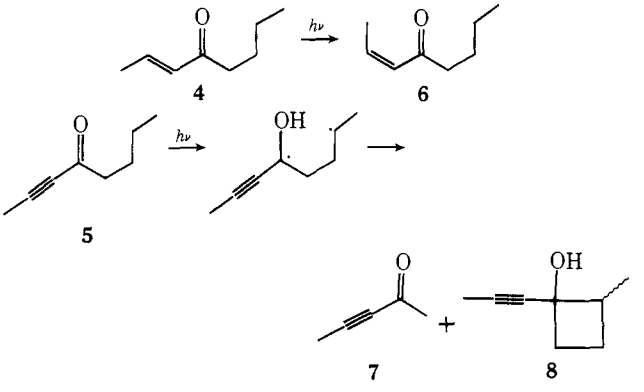
Table I. Photochemical Parameters for Several Ketones

Compound	Φ_{-K}[a]	Φ_{Cb}[b]	Φ_{Cl}[c]	$k_q\tau$[d]	E_T[e]	Ref
2-Hexanone	0.33, 0.5[h]	0.075	0.252	50	(80)[f]	1, 15, 16
Valerophenone	0.42	0.091	0.33	36	74.5	17-19
4	0.49 ± 0.01	0	0	2	(73)[g]	This work
5	0.43 ± 0.03	0.12 ± 0.02	0.32 ± 0.03	16	73.1	This work

[a] Quantum yield for ketone disappearance in benzene. [b] Cyclobutanol formation. [c] β-Cleavage. The value for **7** is based on its calibrated GC response factor. [d] Stern-Volmer quenching constant of Φ_{-K} with piperylene. [e] Triplet energy, kcal mol^{-1}. [f] By analogy with acetone, ref 20. [g] By analogy with cyclic enones, ref 21. [h] In pentane and hexane, respectively.

appeared,[10] we decided that a direct comparison of an ynone with its related enone would be worthwhile. Cyclobutanol formation from an ynone could be a useful synthetic procedure because in contrast to the alkyl phenyl ketones, the product offers various possibilities for further chemical transformations.[11] We have therefore examined the photochemistry of compounds **4** and **5**.



Irradiation of **4**[12] in degassed benzene at 313 nm gave only one product according to GC. Spectral data suggested that it was cis enone **6** and this was confirmed by comparison with the material produced by partial hydrogenation of **5**: (**6**) ir (CCl_4) 3030, 1690, 1625, 930 cm^{-1}; NMR (CCl_4) δ, 0.93 (br t, J = 5.5 Hz, 3 H), 1.43 (m, 4 H), 2.04 (br, dd, J = 5.1 Hz, 3 H), 2.33 (br, t, J = 6.5 Hz, 2 H), 6.03 (br d J = 2 Hz, 2 H). Irradiation of **5**[13] (uv(cyclohexane) λ_{max} 310 nm, ε 27) under the same conditions gave rise to three new GC peaks. The two closely spaced, equal area peaks at long retention time were shown by GC/MS to be isomeric with starting material while the early peak corresponded to loss of propene from **5**. Preparative GC allowed isolation of the isomeric products as a mixture and spectral data quickly pinpointed their structure as the epimers of **8**: ir (CCl_4) 3610, 2240 cm^{-1}; NMR (CCl_4) δ 1.02 (br d, J = 6.5 Hz, 3 H), 1.81, 1.87 (s, 3 H), 1.02-2.6 (br m, 5 H); exact mass 124.0885; calcd for $C_8H_{12}O$, 124.0887. The early GC peak was isolated and shown to be identical with an authentic sample[14] of **7** (ir (CCl_4) 1680, 2230; NMR (CCl_4) δ 1.97 (s, 3 H), 2.17 (s, 3 H)).

It is clear from these results that enone **4** undergoes exclusive geometric isomerization while ynone **5** gives γ-hydrogen abstraction products, in accord with our expectations. Further experiments were aimed at defining the excited state responsible for these photoreactions. Trans-cis isomerization of **4** was hardly quenched with *cis*-piperylene (Table I) and only minor amounts of *trans*-piperylene were formed. Since many enones appear to intersystem cross[6,21,22] and since geometric isomerization is a characteristic triplet process, the excited state involved here is probably a very short-lived triplet. Ynone **5** gives γ-hydrogen alstraction products in high chemical yield and quantum yield from the triplet state. Not only was the reaction quenchable with *cis*-piperylene, but isomerization to trans was readily achieved with **5** as sensitizer. Furthermore, irradiation of **5** in acetone at 313 nm gave the same products and the same quantum yields as direct irradiation.

In view of the similarity in photochemical behavior between **5** and valerophenone (Table I), it was of obvious interest to examine the phosphorescence spectrum of **5**. Strong, nicely structured emission was observed, whose 0-0 band corresponded to a triplet energy of 73.1 kcal mol^{-1} and whose lifetime (MCIP glass −196°) was 2 ms. Thus, the analogy with valerophenone is borne out and the (n,π*) state is implicated as the photoreactive one in **5**.

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Bovine Serum Albumin as a Catalyst. 4. Identification of the Active Site

Sir:

Recently we reported in detail an unusual catalytic enzyme-like property for bovine serum albumin (BSA).[1-3] In the pH range of 7-9 BSA accelerates by a factor of ca. 10^4 the rate of decomposition of the Meisenheimer complex, 1,1-dihydro-2,4,6-trinitrocyclohexadienate (MC). It was found from inhibition studies that the active site of the protein is also the high affinity binding site for pyridoxal 5′-phosphate (PP).[2]

In 1971 Anderson et al. determined the sequence of residues in the PP binding site of BSA: Ser-Leu-Phe-Glu-Lys-Pro-Lys-Lys.[4] The use of borohydride reduction of the Schiff base followed by amino acid analysis indicated that the penultimate lysine in the above sequence was the site of binding with PP. It seemed reasonable to us that this sequence contained the

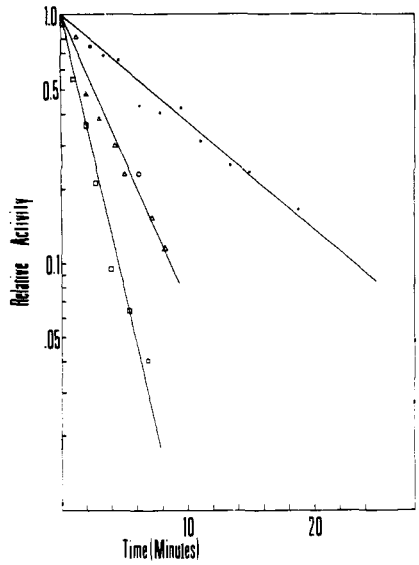

Figure 1. First-order plot illustrating the loss of catalytic activity of IA-BSA as a function of time at different pH values at room temperature. The concentration of IA-BSA was ca. 40 μM, and the concentration of FITC was ca. 80 μM. Periodically small aliquots (ca. 50 μl) were removed from the incubation mixture and assayed for activity in 3 ml of buffer using our "standard assay" conditions.[2] The three lines, in order of *increasing* rate, represent pH 8.0, 8.83, and 9.46 in either 0.05 M phosphate (pH 8.0) or 0.02 M borate (pH 8.83 and 9.46). The straight lines drawn through the data correspond to first-order rate constants of ca. 0.10, 0.26, and 0.51 min^{-1}, respectively, in order of increasing pH.

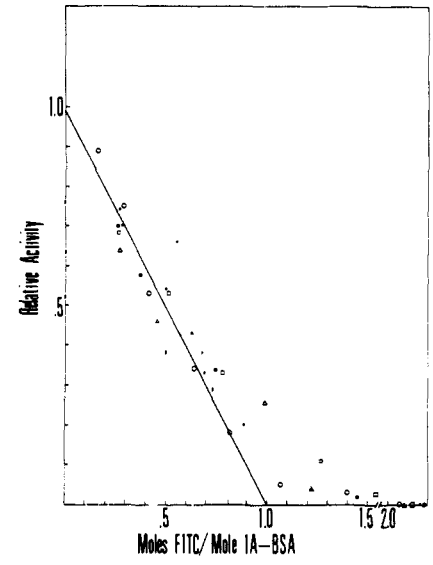

Figure 2. Correlation of the stoichiometry of labeling of IA-BSA by FITC and the loss of catalytic activity. Different symbols represent independent experiments. Various molar ratios of FITC to IA-BSA were incubated for ca. 2 h at either pH 8.2 or 9.0 in 0.02 M borate at room temperature and then dialyzed exhaustively at pH 3.0 in the cold. They were then assayed for catalytic activity, and the degree of coupling was determined from the optical density of aliquots of the solutions at 491 nm in 0.1 M NaOH. The molar extinction coefficient for FITC coupled to BSA was taken as 75 900 under these conditions.[7] The protein concentration was determined on controls, run under identical conditions, in the absence of FITC. The straight line illustrates the expected results if only the catalytic site were to react with FITC, and complete loss of activity were to result.

active site residues (presumably lysines) responsible for the unusual catalytic activity we have described.

However, in a classic piece of work, Brown[5] has now completed the sequencing of the entire protein, and his results indicate that no such sequence of residues is present in BSA. This has led us to search for other active-site directed reagents in an effort to delineate those residues which are in fact responsible for the catalysis. We have found that fluorescein isothiocyanate (FITC) reacts rapidly and stoichiometrically with BSA and destroys its catalytic activity. The results of our studies, combined with Brown's sequence data, indicate that lysine 220 must be at the active site of the protein.

IA-BSA[6] was prepared as described previously;[2] assays of its catalytic activity toward MC followed our published protocols. FITC (isomer I) was obtained from Sigma and was prepared fresh daily by dissolving it in dimethylformamide to make solutions of ca. 0.2 M concentration which were assayed spectrophotometrically using the published molar extinction coefficient of the molecule.[7] The effect of FITC on the catalytic activity of IA-BSA was determined by adding small aliquots (<1% final volume) of the stock solution of FITC to solutions of IA-BSA which were subsequently assayed for catalytic activity as a function of the time of incubation with FITC.

The reaction between FITC and IA-BSA leads to a rapid loss of catalytic activity (Figure 1). The kinetics of inactivation of IA-BSA by FITC are first order, and show a saturation phenomenon. For example, at pH 8.2, the rate of loss of activity is the same for initial concentrations of FITC between 80 and 320 μM and for initial concentrations of IA-BSA of ca. 40 μM. This suggests that the mechanism of inactivation involves the rapid formation of a noncovalent complex between FITC and IA-BSA, followed by a slower nucleophilic attack of a specific residue of the protein (presumably lysine, see below) on the FITC to form the covalent thiocarbamyl derivative. In the product the active site lysine is obviously blocked, and thus the derivative will be catalytically inactive. As would be expected, the rate of loss of activity increases at higher pH (Figure 1). This is reasonable, as we have previously shown[2] that the BSA-catalyzed reaction requires that a lysine of pK_a 8.38 be unprotonated, and presumably this group must also be unprotonated in order to react with FITC.

The stoichiometry of the labeling reaction indicates that modification of a single residue destroys the catalytic activity of the protein (Figure 2). The results do suggest that other residues also react with FITC, but it is clear that the primary site of reaction is also involved in the catalytic mechanism of the protein.

Further verification that the lysine which reacts rapidly with FITC is in fact at the catalytic active site of the protein derives from protection experiments using PP. Under the standard conditions of our experiment (FITC at 80 μM, IA-BSA at 40 μM, pH 8.2) 2 h of incubation of IA-BSA with FITC leads to complete loss of catalytic activity. However, if the IA-BSA is preincubated with stoichiometric quantities of PP (1 mol per mole of IA-BSA) and then the protein is allowed to react with FITC for about 2 h, about 45% of the catalytic activity of the protein is recovered after the PP and unreacted FITC are removed by exhaustive dialysis in the cold at pH 3.0.[8] Controls in which the PP was omitted had no detectable activity after dialysis. This experiment provides strong evidence that the PP binding site is the site which is labeled by FITC.

Andersson et al.[9] have identified the residues present in the site in BSA which is preferentially labeled by FITC:

Leu·Ser·Gln·Lys-Phe-Pro-Lys
FITC

The lysine is at position 220 in Brown's sequence.[5] A reexamination of the array of residues reported by Anderson et al.[4] for the PP binding site indicates that most of the residues reported in their sequence are in fact present (though in different order) in the FITC binding site. In fact, in the entire sequence of the protein the sequence of residues in the FITC site is most similar to Anderson's reported sequence for the PP binding site. In view of this evidence, coupled with our observations described above, it is most probable that a few small errors were

made in the sequence work on the PP site, and it is likely that the correct sequence for this site is the same as that for the FITC site.

We have previously reported that HSA has little, if any, catalytic activity with respect to the decomposition of MC, as compared to BSA.[2] Brown's sequence work on HSA[5] indicates that the critical lysine 220 in BSA is replaced by an arginine in the homologous position (residue no. 221) in HSA. This provides a clear molecular proof for the critical importance of lysine 220 in the catalytic mechanism of BSA. It is likely that other albumins which do have this unusual activity will also have lysine at the homologous position.

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First Example of Photochemical Allowed $[4\pi + 2\sigma + 2\sigma]$ Cycloaddition[1]

Sir:

Many more examples showing the application of the generalized selection rules for pericyclic reactions have been reported.[2,3] It is surprising, however, that the photochemical allowed $[4\pi + 2\sigma(2\pi) + 2\sigma(2\pi)]$ cycloaddition has no precedent in chemical literature.[4] Quadricyclane seems to be a good model for this purpose.[5]

We have investigated a photochemical reaction between aromatic hydrocarbons and quadricyclane and thereby found the first concrete evidence for photochemical allowed $[4\pi + 2\sigma + 2\sigma]$ cycloaddition between a 4π system and a pair of strained σ bonds.

Murov and Hammond[6] have reported that quadricyclane is a very effective quencher of the fluorescence of aromatic hydrocarbons such as anthracene and naphthalene, and suggested that the electronic energy of the aromatic hydrocarbons is efficiently transferred to vibrational energy of quadricyclane followed by isomerization to norbornadiene. Solomon et al.[7] have described that the fluorescence quenching of aromatic hydrocarbons by quadricyclane could proceed via a charge-transfer complex. In spite of these efforts, the possibility of chemical product formation has not been excluded, since Yang et al.[8] and authors[9] have shown that some aromatic hydrocarbons undergo highly efficient photocycloadditions with several cyclic diene and trienes resulting in the chemical product formations through a significant pathway for the decay of the exciplexes.

When a solution of anthracene (**1**) and a large excess of quadricyclane (**2**) in benzene was irradiated with a high-pressure mercury lamp (100 W) in a Pyrex vessel at 20° for 6 h, anthracene was rapidly consumed. After removal of the anthracene photodimer **3** (65%) precipitated during the irradiation, careful chromatography of the photolysis mixture on silica gel using benzene–*n*-hexane as eluent gave a 1:1 adduct **4** (mp 157–160°) in 45–50% yield. Interestingly, similar irradiation under the same conditions in the presence of methylene bromide resulted in the quantitative formation of the dimer **3**, conversion of norbornadiene (20%), and recovery of **2** (80%), and no 1:1 adduct was detected. The structure of the adduct **4** was deduced from elemental analysis (*Anal.* Calcd for C, 93.69; H, 6.31. Found: C, 93.43; H, 6.57), mass spectrum (M^+ 270), and its NMR data: the spectrum in CCl_4 exhibits signals of two bridged methylene protons at δ −0.19 (d, 1 H, syn H-13, J = 10.5 Hz), and 0.68 (d, 1 H, anti H-13, J = 10.5 Hz), methine protons at δ 2.02 (d, 2 H, H-2 and H-7, J = 1.0 Hz) and 2.41 (d, 2 H, H-3 and H-6, J = 1.8 Hz), benzylic methine protons at δ 4.04 (d, 2 H, H-1 and H-8, J = 1.0 Hz), olefinic protons at δ 6.03 (d, 2 H, H-4 and H-5, J = 1.8 Hz), and aromatic protons at δ 6.92–7.18 (m, 8 H). An appearance of the higher bridged methine proton signal was attributable to the anisotropy of benzene moiety which was to be expected for the exo isomer by molecular models. Thus, the adduct was concluded to be exo$[4\pi + 2\sigma + 2\sigma]$cycloadduct **4**. It is to be noted that both the thermal and photochemical reactions of **1** and norbornadiene (**5**) gave no 1:1 adduct even under more drastic conditions. The external heavy-atom effect as described above suggests a singlet mechanism for the addition process. These results support the idea that the formation of photoproduct **4** may be rationalized as a concerted photoallowed $[{}_{\pi}4_s + {}_{\sigma}2_s + {}_{\sigma}2_s]$ addition between singlet excited anthracene and quadricyclane.

On the other hand, the fluorescence of anthracene and naphthalene was efficiently quenched by **2** and linear Stern–Volmer plots were obtained for the quenching reactions. The rate constant for the quenching reaction of anthracene (3.1×10^9 l. mol^{-1} s^{-1}) is about the same as that of naphthalene (3.2×10^9 l. mol^{-1} s^{-1}). However, when a solution of naphthalene (**6**) and **2** in benzene was irradiated under the same conditions through a Corex filter, extensive isomerization of **2** to norbornadiene (**5**) was observed. One interesting implication comes from consideration of the fact that the chemical product could not be detected from quenching of naphthalene by **2** in comparison with the case of anthracene. It might be explained

Scheme I