sucrose, at the same molarity, did not produce this enhancement (Figure 2, curve 2). It is of interest to note that N-acetyl-D-galactosamine produced an enhancement similar to that seen with NAG (Figure 2, curve 1). These findings indicate that the exposed aromatic residues in the substrate-binding site of lysozyme contribute to the optical activity observed and that *their orientation is probably affected by substances binding at this site.*

Study of the effect of sodium dodecyl sulfate and of ethylene glycol on the CD of lysozyme (Figure 3) confirmed and extended our earlier findings. Ethylene glycol increased the magnitude of the CD bands in the region 280–300 mμ, but had no influence on the negative band centered near 262 mμ. The CD of lysozyme in 50% ethylene glycol resembles closely that obtained in the presence of NAG. Presumably, in aqueous ethylene glycol solution, the orientation of the optically active aromatic chromophores is similar to that obtained in the presence of NAG. Perhaps in the case of both NAG and ethylene glycol the enhancement of optical activity arises as a consequence of displacement of water from the substrate-binding crevice,[4] permitting some reorientation of the aromatic residues. Sodium dodecyl sulfate (Figure 3, curve 3) completely eliminated the aromatic CD bands. This result is in total accord with our earlier optical rotatory dispersion findings.[2] In addition, however, sodium dodecyl sulfate greatly diminished the magnitude of the negative 262-mμ band. A similar observation was reported by Beychok[7] for the effect of urea on the CD of insulin. From our earlier optical rotatory dispersion[2] and these CD data, the detergent appears to disorganize profoundly all those regions of lysozyme not in α-helical segments.

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The Mechanism of Direct *cis–trans* Photoisomerization of the Stilbenes

Sir:

Hitherto available data on the *cis–trans* photoisomerization of the stilbenes can be accommodated by either of two mechanisms: (1) the Lewis mechanism,[1] which assumes that $S^1 \rightarrow S^0$ radiationless conversion produces a freely rotating ground state; (2) the Förster mechanism,[2–7] in which the stilbene isomers lose their identity upon $S^1 \rightarrow T^1$ crossing to interconvertible or common triplet states.

Preference for the triplet-state mechanism is based on the Kassel–Rice theory, which predicts that energy available upon internal conversion to the ground state is rapidly distributed among various vibrational modes. The Lewis mechanism is, therefore, not expected to compete with loss of vibrational excitation to solvent.[8]

Two findings are not easily compatible with the triplet mechanism: (1) the frequency factor (10^{12} sec^{-1}) for the main path leading to isomerization of *trans*-stilbene is larger than would be expected for a spin-forbidden process;[3,6] (2) the azulene effect on the sensitized isomerization is accounted for by eq 1.[7]

$$t^{T_1} + Az^{S_0} \longrightarrow t^{S_0} + Az^{T_1} \qquad (1)$$

However, azulene's effect on the direct photoisomerization cannot be quantitatively accounted for by eq 1.[7] The discrepancy is shown in Figure 1, where the dependence of the ([*trans*]/[*cis*])$_S$ ratio at the photostationary state on azulene concentration is compared to the straight line expected for the triplet mechanism.

We present data which show that the discrepancy in Figure 1 is best interpreted as evidence against the involvement of triplet states in the direct photoisomerization of the stilbenes. We consider the following kinetics for the Lewis mechanism

$$c^{S_0} \xrightarrow[3130\ \text{A}]{h\nu} c^{S_1} \qquad (2)$$

$$t^{S_0} \xrightarrow[3130\ \text{A}]{h\nu} t^{S_1} \qquad (3)$$

$$t^{S_1} \longrightarrow t^{S_0} + h\nu \qquad (4)$$

$$t^{S_1} \longrightarrow at^{S_0} + (1 - a)c^{S_0} \qquad (5)$$

$$c^{S_1} \longrightarrow \beta t^{S_0} + (1 - \beta)c^{S_0} \qquad (6)$$

$$t^{S_1} + Az^{S_0} \longrightarrow t^{S_0} + Az^{S_1} \qquad (7)$$

Energy transfer from c^{S_1} to azulene is neglected because the lifetime of c^{S_1} is expected to be shorter than that of t^{S_1}.[1,4,9] The self-quenching step (8) is neglected

$$t^{S_1} + t^{S_0} \longrightarrow 2t^{S_0} + \text{energy} \qquad (8)$$

for low concentrations of *trans*-stilbene.[7] The mechanism yields stationary-state relationship 9 where

$$\left[\frac{[t^{S_0}]}{[c^{S_0}]}\right]_S = \left[\frac{\epsilon_c}{\epsilon_t}\right]\left[\frac{\beta}{1 - \alpha}\right]\left[1 + \frac{k_4}{k_5} + \frac{k_7[\text{Az}]}{k_5}\right] \qquad (9)$$

ϵ_c and ϵ_t are extinction coefficients of *cis*- and *trans*-stilbene at the exciting wavelength. The initial slope to intercept ratio of curve 1 in Figure 1 gives $k_7/(k_4 + k_5) \leq 40\ M^{-1}$.

trans-Stilbene fluorescence can be induced by β-ray radiation.[10,11] A scintillation counting method was developed to evaluate singlet excitation transfer from *trans*-stilbene to azulene (eq 7). The scintillation system consisted of benzene-^{14}C as solvent and β-ray source, *trans*-stilbene as fluor, and azulene as

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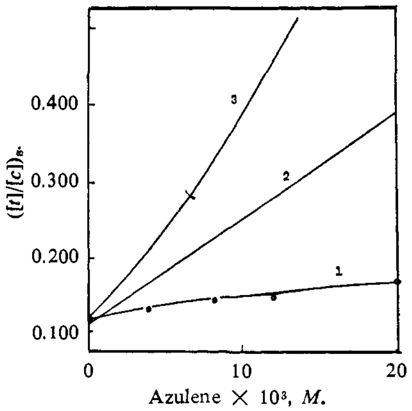

Figure 1. Curve 1: observed effect of azulene on unsensitized isomerization of stilbene;[7] curve 2: expected behavior for triplet mechanism; curve 3: expected behavior for triplet mechanism including singlet energy transfer (eq 7).

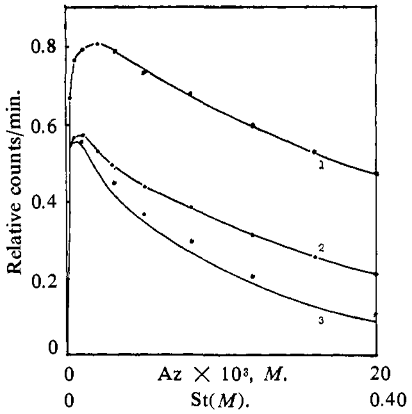

Figure 2. Curve 1: counting efficiency as a function of *trans*-stilbene concentration; curve 2: azulene outside inner ampoule (trivial quenching); curve 3: calculated using eq 11 for azulene within inner ampoule. Closed circles are experimental points.

quencher. The *trans*-stilbene and benzene-^{14}C were sealed into a degassed ampoule, which was centered within a larger vial[12] and surrounded with benzene. To differentiate between radiationless singlet excitation transfer (eq 7) and absorption of *trans*-stilbene fluorescence by azulene ("trivial" transfer) parallel sets of azulene experiments were conducted. In one set the azulene was included in the inner ampoule and in the other the azulene was placed in the outer benzene solution. In the latter set, the azulene concentration was adjusted so that the total cross-sectional optical density was identical for azulene outside or inside the inner ampoule. Representative results are shown in Figure 2. The difference between the points for curve 2 (azulene outside) and curve 3 (azulene inside) is attributed to radiationless singlet energy transfer (eq 7).[13]

The fact that the 1,3-pentadienes reduce the counting efficiency of several scintillation systems was interpreted to indicate that most of the energy from the β-ray resides initially in triplet states which produce fluorescent singlet states by triplet–triplet annihilation.[15] Therefore, two additional mechanisms for azulene quenching were evaluated. The first, quenching of solvent triplets (eq 10), was considered improbable

$$\text{benzene}^{T^1} + \text{Az}^{S^0} \longrightarrow \text{benzene}^{S^0} + \text{Az}^{T^1} \qquad (10)$$

because azulene was present at much lower concentration than *trans*-stilbene. It was rejected since a mixture of the 1,3-pentadienes was shown not to quench *trans*-stilbene scintillation when included in concentrations up to and exceeding the highest azulene concentration by a factor of five. The possibility that azulene quenches *trans*-stilbene triplets (eq 1) which might have reached the fluorescent state by triplet–triplet annihilation was rejected because *cis*-stilbene shows no scintillation. Since conversion of *cis* triplets to *trans* triplets is fast,[7] absence of emission from *cis*-stilbene shows that the number of stilbene triplets which reach S^1 is negligible.

Since fluorescence intensity is proportional to counting efficiency, eq 4–8 lead to steady-state expression 11, where X is the ratio of counting efficiency for

$$X = 1 + \frac{k_8[t^{S^0}]}{k_7[\text{Az}^{S^0}]} + \frac{k_4 + k_5}{k_7[\text{Az}^{S^0}]} \qquad (11)$$

trans-stilbene alone to the difference between counting efficiencies for azulene outside and azulene inside the inner ampoule. The decrease in counting efficiency at higher *trans*-stilbene concentrations (curve 1, Figure 2) is attributed to step 8. As expected, the plot of (relative counts/min)$^{-1}$ *vs.* *trans*-stilbene concentration is linear for the high concentration range of the data in curve 1, Figure 2. The ratio of the slope to the extrapolated intercept of this plot yields $k_8/(k_4 + k_5) = 3\ M^{-1}$. This value, the ratio $k_7/(k_4 + k_5) = 40\ M^{-1}$ from the isomerization experiments, and eq 11 were used to calculate curve 3 in Figure 2. The calculated curve agrees well with experimental points for azulene within the inner ampoule. Equation 11 was used successfully to predict the effect of eq 7 for similar experiments where [St]/[Az] = 50 and for a set of experiments in which *trans*-stilbene concentration was held constant at $10^{-1}\ M$ and azulene varied from 10^{-5} to $8 \times 10^{-3}\ M$.

We have shown that radiationless singlet excitation transfer (eq 7) quantitatively accounts for the azulene effect on the direct photoisomerization. We therefore conclude that, contrary to prevalent views,[2-8] the Lewis mechanism for the isomerization obtains. The high frequency factor[3,6] for the crossing into the state from which isomerization occurs is now reasonable, since the process is $S^1 \rightarrow S^0$ internal conversion. For *trans-p*-bromostilbene the main path leading to isomerization is an unactivated process.[3,6] If this process is heavy-atom-enhanced intersystem crossing, the azulene effect on the direct isomerization of *trans-p*-bromostilbene should correspond to line 2 in Figure 1. We intend to investigate this system.

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[12] Packard 24-mm neck vials were used as well as a Packard Tri-Carb scintillation counter, courtesy of Professor R. J. Light of this department.

[13] The extent of trivial quenching is, qualitatively, as would be expected on the basis of the absorption spectrum of azulene[14] in the frequency region of *trans*-stilbene fluorescence.[3]

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Direct Distinction between Ions and Ion Pairs in Electron-Transfer Reactions by Means of Electron Spin Resonance[1]

Sir:

Electron spin resonance (esr) has been shown to be uniquely suitable for measuring the rates of fast electron-transfer reactions between radical ions and neutral molecules in solution.[2] Depending on the nature of the solvents and the counterions, these radicals may exist as free ions, ion pairs,[3] or a mixture of both. Accordingly, electron-transfer reactions in the case of aromatic anion radicals can be represented by

$$R + R^- \rightleftarrows R^- + R \qquad (1)$$

$$R + M^+R^- \rightleftarrows M^+R^- + R \qquad (2)$$

where R is the neutral molecule, R^- the anion radical, and M^+ the alkali metal ion. In general it has not been possible to distinguish between reactions 1 and 2 since measurement of their individual rates requires the observation of distinctly different esr spectra for the two species. Except when M has an appreciable magnetic moment, *e.g.*, sodium, most of the ion-paired radicals show esr spectra which have unresolved metal splittings. The only measurements of rates which distinguish ions and ion pairs are those reported by Zandstra and Weissman for the naphthalene–naphthalenide system in tetrahydrofuran (THF) and similar solvents using sodium as the reducing agent.[4] The large sodium splittings and the detectable concentrations of both the free and ion-paired species enabled the observation of two overlapping esr spectra over a wide range of temperatures.

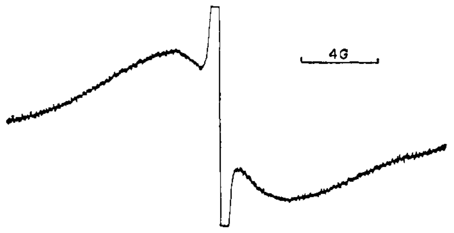

Figure 1. Esr first derivative spectrum obtained when naphthalene (1.4 *M*) was reduced with potassium in tetrahydrofuran. The clipped center peak indicates an off-scale reading.

We have been able to distinguish the free ion from the ion pair in certain cases and to separately measure the rates of their oxidation–reduction reactions by making use of the hitherto unexploited "fast exchange limit." [5] In this regime the entire spectrum for each species collapses to a single line of Lorentzian shape or possibly a set of equally spaced lines for atom transfers,[6] and the equation of Piette and Anderson can conveniently be used to determine the second-order rate constant for the exchange reaction.[7] When rewritten for this situation their equation becomes $k = 2.04 \times 10^7 \nabla/\Delta H[\mathrm{R}]$, where ∇ is the second moment (in gauss^2) of the spectrum of the species of interest in the absence of exchange, ΔH is the line width[8] (in gauss) corrected for modulation, natural line width, etc., and [R] is the concentration (in moles/liter) of the neutral species. Of course, the measurement of more than one rate in a single experiment requires that the rates be well separated. A typical experimental curve is shown in Figure 1 to illustrate the separability of the species in our study.

By the above procedure rate constants have been measured for naphthalene in reactions 1 and 2 using THF and 1,2-dimethoxyethane (DME) as solvents and potassium and sodium as reducing agents. A summary of the results is given in Table I along with rate constants measured by Weissman and his co-workers for comparison. In the Na–DME system at high naphthalene concentrations, only one line was observed from −30 to +40°. When potassium was used as the reducing agent in DME, a single line was also observed; however, in THF two lines of appreciably different widths were found (see Figure 1). A second broad line was also observed in the K–DME system after the addition of potassium chloride. Finally, for the Na–THF combination we obtained a single line superimposed on a broad uncollapsed spectrum. As expected the strongly collapsed lines were always rigorously Lorentzian, thus confirming the fast exchange limit. The greatest errors

Table I. Rate Constants for Naphthalene–Naphthalenide Systems at 23°

Metal ion	Sol-vent	Species	Fast exchange limit, *k*, M^{-1} sec^{-1}	Previous work,[a] *k*, M^{-1} sec^{-1}
Na	DME	Ion	$(1.6 \pm 0.2) \times 10^9$	$(1 + 3 - 0.7) \times 10^9$
Na	THF	Ion[b]	$(2 \pm 1.5) \times 10^9$	$(8 \pm 4) \times 10^7$
		Ion pair	$<3 \times 10^7$	$(1.8 \pm 0.6) \times 10^7$
K	DME	Ion	$(2.2 \pm 0.4) \times 10^9$	
		Ion pair[c]	$(1.0 \pm 0.5) \times 10^8$	$(7.6 \pm 3) \times 10^7$
K	THF	Ion	$(3.0 \pm 0.5) \times 10^9$	
		Ion pair	$(6 \pm 2) \times 10^7$	$(5.7 \pm 1) \times 10^7$

[a] Obtained from ref 2 or calculated from the data in ref 4. [b] This measurement was hampered at the fast exchange limit by the presence of an uncollapsed background. [c] At the fast exchange limit this rate was measured in the presence of a 5 m*M* concentration of KCl.

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[8] Here the line width is taken to mean the separation between the extrema in a first derivative spectrum.