1,2-diaxial-trans ring juncture will be too strained to exist under normal conditions. It is reasonable to assume that most photochemical processes are faster than ring inversion in cyclohexane. Therefore, only one of the two conformations of the *cis*-ketones can undergo isomerization without ring inversion (see Chart I), *i.e.*, the one with an axial $\alpha$-C—C=O (**1c**), which will give **1b** in the normal 1,2-diequatorial conformation. The cleavage of an axial bond in these systems will relieve more steric strain at the transition state than the cleavage of the corresponding equatorial bond, and therefore will proceed with less activation energy or at a faster rate. The photochemistry of these ketones occurs mostly from the singlet excited state, indicating that the rate of $\alpha$ cleavage from the singlet excited state is at least competitive with that of intersystem crossing even with the $\alpha$-C—C=O group in the equatorial position. The rate of intersystem crossing of *tert*-alkyl ketones is relatively independent of their structure.[3] Therefore, it is not surprising that the rate of $\alpha$ cleavage of an axial $\alpha$-C—C=O bond will be appreciably faster than the rate of intersystem crossing, and that the $\alpha$ cleavage in **1c** with subsequent ring closure to **1b** occurs virtually all from the singlet excited state. The variation of intersystem crossing efficiencies of these compounds may also be related to the competition between the rates of intersystem crossing and $\alpha$ cleavage. The decalones are thermodynamically more stable than the hydrindanones, and the *cis*-decalone **2a** is more stable than the *trans*-decalone **2b**.[14] The difference in stability among these compounds may be attributed to variation in internal strain, and the relative strain in their excited states may parallel the strain in their ground state. The rate of $\alpha$ cleavage will decrease as the internal strain of these molecules decreases; therefore, the efficiency of intersystem crossing of **1a**, **1b**, **2b**, and **2a** will increase in the order observed.

The quantum efficiencies of isomerization and chemical nonradiative decay of the excited states of these compounds will depend on the stereochemistry of ring closure of the biradical intermediate (**4** from **1a** or **1b**; see Chart I). The intermediate, which is a derivative of the cyclohexyl radical, will give the *cis*-ketone by axial ring closure and the *trans*-ketone by equatorial ring closure. The stereochemistry of reactions of cyclohexyl radicals is known to occur preferentially from the axial side than from the equitorial side.[15] The experimental observation that photoisomerization occurs much more efficiently from *trans*-ketones than from *cis*-ketones, *e.g.*, $\phi_{1b\rightarrow 1a} = 0.46$, $\phi_{1a\rightarrow 1b} = 0.02$, may be readily explained by preferential ring closure of the biradical intermediate from the axial side.

In conclusion, we observed that these ketones (**1a**, **1b**, **2a**, and **2b**) readily undergo photoisomerization from either excited state. The results imply that inefficiency of the type I process of aliphatic ketones in solution may be due to the cage recombination of initially dissociated radicals, and such a process may occur from both the singlet excited and the triplet state.

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(13) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 279.
(14) A. Ross, P. A. S. Smith, and A. S. Dreiding, *J. Org. Chem.*, **20**, 905 (1955).
(15) H. L. Goering, D. I. Relyea, and D. W. Larsen, *J. Amer. Chem. Soc.*, **78**, 348 (1956); F. R. Jensen, L. H. Gale, and J. E. Rodgers, *ibid.*, **90**, 5793 (1968); J. G. Traynham, A. G. Lane, and N. S. Bhacca, *J. Org. Chem.*, **34**, 1302 (1969).

(16) University Fellow, 1968–1969; UniRoyal Fellow, 1969–1970; William Raney Harper Fellow, 1970–1971.

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## Fluorenone-Photosensitized Isomerization of *trans*-Stilbene. Inefficiencies both in Intersystem Crossing and in Triplet Excitation Transfer

*Sir:*

The quantum yield of isomerization of *trans*-stilbene sensitized in benzene by fluorenone has been reported[1] to be 0.41. This quantum yield is markedly lower than the quantum yield, $\phi_{ST}\alpha$, predicted from the previously measured[2] intersystem crossing quantum yield $\phi_{ST} = 0.93$ for fluorenone in benzene and the known[1] decay fraction $\alpha = 0.59$ for stilbene triplet → *cis*-stilbene. Since other quantum yields reported at the same time[1] have since been shown to be somewhat low,[3] we have repeated this measurement. Our results, surprisingly, indicate that 0.41 is essentially correct. We have performed additional experiments which shed light on the source of this unusual quantum inefficiency. At this time we (1) report the intersystem crossing quantum yields $\phi_{ST}$ of fluorenone in several solvents; (2) substantiate the previously reported[4] large solvent effect on the rate constant, $k_{ST}$, for intersystem crossing in fluorenone; (3) demonstrate that internal conversion ($k_{IC}$) can be significant in the deactivation of fluorenone singlet; and (4) present results which appear to eliminate several possible alternative bimolecular processes and leave decay from a triplet exciplex as the most probable source of the inefficiency in the fluorenone triplet–*trans*-stilbene interaction.

Quantum yields of production of *cis*- and *trans*-diacetoxyethylene (DAE) in the fluorenone-sensitized fragmentation of *trans*-7,8-diacetoxybicyclo[4.2.0]octa-2,4-diene[5] were monitored at 366 nm, relative to parallel and otherwise identical experiments in which benzophenone ($\phi_{ST} \equiv 1.00$) was used as sensitizer. The relative quantum yields (Table I) thus give $\phi_{ST}$ for fluorenone in the three solvents employed. The value of essentially unity in cyclohexane clearly agrees with the observed[4] low fluorescence yield and the interpretation[4] that $k_{ST}$ is largest here; furthermore, it indicates that there is no

(1) G. S. Hammond, *et al.*, *J. Amer. Chem. Soc.*, **86**, 3197 (1964).
(2) A. A. Lamola and G. S. Hammond, *J. Chem. Phys.*, **43**, 2129 (1965).
(3) H. A. Hammond, D. E. DeMeyer, and J. L. R. Williams, *J. Amer. Chem. Soc.*, **91**, 5180 (1969).
(4) (a) R. A. Caldwell, *Tetrahedron Lett.*, 2121 (1969); (b) J. B. Guttenplan and S. G. Cohen, *ibid.*, 2125 (1969); (c) L. A. Singer, *ibid.*, 923 (1969).
(5) R. A. Caldwell, *J. Org. Chem.*, **34**, 1886 (1969).

Table I

| Solvent[a] | $\phi_{ST}$(fluorenone) | $k_{ST}$[b] | $k_F$[b] | $k_{IC}$[b] | $\phi_{t\to c}$ (%)[c] Stilbene | $\phi_{t\to c}$ (%)[c] α-Cyanostilbene |
|---|---|---|---|---|---|---|
| Cyclohexane | 1.03 ± 0.01 | 660 | | | 0.47 ± 0.015 (78) | 0.50 ± 0.01 (82) |
| Benzene | 0.93 ± 0.01 | 31 | 0.4 | 2 | 0.43 ± 0.015 (78) | 0.48 ± 0.01 (85) |
| Acetone | 0.77 ± 0.01 | 8.5 | 0.3 | 2 | 0.37 ± 0.015 (82) | 0.39 ± 0.01 (85) |

[a] Solvents dried over Molecular Sieves (Linde 4A) prior to use. [b] All $k$'s $\times 10^{-7}$ $M^{-1}$ sec$^{-1}$. [c] Percentages given refer to $\phi_{t\to c}/\phi_{ST}\alpha$, where $\alpha$ = 0.59 for stilbene and 0.60 for α-cyanostilbene. The value for α-cyanostilbene was derived from photostationary-state studies using high-energy sensitizers (anthraquinone and Michler's ketone).

inherent quantum inefficiency in the triplet fluorenone–COTDA interaction. The value of 0.93 in benzene is in excellent agreement with a previously reported value.[2] On this basis, the value of 0.77 in acetone clearly represents a significant deviation from unity. Previously reported absolute[4c] and relative[4a] quantum yields for fluorenone fluorescence allow calculation of $\phi_F$ in acetone as only *ca.* 0.03; it is thus clear that *some 20% of fluorenone singlet decays by internal conversion in acetone.*

Based on the reported[4a] singlet lifetimes, we calculate from $\phi_{ST}$ and the fluorescence[4a,c] quantum yields the rate constants $k_F$, $k_{ST}$, and $k_{IC}$ for fluorenone where possible in each solvent. The radiative lifetimes are in good agreement with the estimate of $k_F = 2\text{–}3 \times 10^6$ sec$^{-1}$ calculated[6] from the absorption spectrum. The constancy of $k_{IC}$ in benzene and acetone suggests that the internal conversion process is, in these cases at least, not related to solvent quenching of $S_1$.[7] This is the first aromatic ketone, to our knowledge, which undergoes simple internal conversion from $S_1$. The rate constant $k_{IC}$ is comparable to estimates[8] for $\pi$–$\pi^*$ aromatic hydrocarbons of comparable excitation energy. The $k_{ST}$ values which we calculate fully substantiate the previously indicated solvent dependence, thought to be due to solvent shifts in the positions of the n–$\pi^*$ and $\pi$–$\pi^*$ states.[4]

Knowledge of the $\phi_{ST}$ values as a function of solvent permits a study of inefficiency in the bimolecular quenching process involving stilbene. Quantum yields for fluorenone-sensitized isomerization of *trans*- to *cis*-stilbene were studied at 405[9] and 366 nm, and quantum yields for fluorenone-sensitized trans-to-cis isomerization of α-cyanostilbene[10] were studied at 405 nm, in outgassed cyclohexane, benzene, and acetone (Table I). We observe essentially the same behavior in all cases; inefficiencies in $\phi_{t\to c}/\phi_{ST}\alpha$ range from 15 to 22%. No significant trend with solvent polarity is observed, and the cyano substituent causes only slight changes. It is thus clear that whatever is responsible for the inefficiency does not involve extensive charge transfer, surely not to the extent that we have observed in other triplet ketone–olefin systems.[11] The absorption spectrum of fluorenone in benzene is insensitive to even high concentrations of stilbene, and the fluorescence of fluorenone in benzene was not quenched by either stilbene or α-cyanostilbene; ground-state complexes and singlet quenching are unimportant.[12] Finally, an experiment with *trans*-α,β-dideuteriostilbene[13] in benzene revealed no isotope effect on the quantum yield. Since excitation transfer shows a direct isotope effect and direct biradical formation is expected to show an inverse effect,[14,15] we would have expected a lowering of the quantum yield if direct biradical formation were occurring. We consider the result significant, though it would be conceivable that the effect is small enough to be masked by experimental error. In any case, we have previously[11,15] presented evidence suggesting that direct biradical formation is unlikely in ketone–olefin photochemistry, particularly in reactions such as these, where rate constants[16] exceed $10^9$ $M^{-1}$ sec$^{-1}$.

The most attractive explanation we have found for the bimolecular inefficiency is expressed in the scheme below; here $Ft^3$ represents a triplet excitation-sharing complex (exciplex) between fluorenone (F) and the trans olefin (t).

$$F^3 + t \xrightarrow{k_X} Ft^3$$

$$Ft^3 \xrightarrow{k_{ET}} F + t^3 \longrightarrow \alpha C + (1 - \alpha)t$$

$$Ft^3 \xrightarrow{k_Q} F + t$$

Our data of course do not rule out the possibility that two independent, bimolecular elementary reactions (excitation transfer and quenching, respectively) are responsible. In this view, quenching would involve a reaction occurring at 15–20% of the excitation transfer rate,[16] *i.e.*, *ca.* $6 \times 10^8$ $M^{-1}$ sec$^{-1}$. Our main reason for preferring the exciplex picture is the difficulty of visualizing a process this fast for the loss of *ca.* 50 kcal of triplet excitation unless it involves significant interaction between the $\pi$ systems, *e.g.* prior complex formation. Furthermore, the constancy of the ratio of excitation transfer to quenching, as solvent and substituent are varied, suggests an intimate relationship. It may be pertinent that the triplet excitation energies of fluorenone (53 kcal/mol) and *trans*-stilbene (50 kcal/mol) are well matched, a situation expected to produce maximal stabilization of excitation-sharing complexes.

(6) N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, New York, N. Y., 1965, p 48.

(7) We have observed both low fluorescence yields and low intersystem crossing yields for fluorenone in ethanol, suggesting that some chemical quenching process by the protic solvent occurs. See, for example, S. G. Cohen, M. D. Salzman, and J. B. Guttenplan, *Tetrahedron Lett.*, 4321 (1969).

(8) W. Siebrand and D. F. Williams, *J. Chem. Phys.*, **49**, 1860 (1968).

(9) Actinometry with 0.025 *M* potassium ferrioxalate, $\phi(Fe^{2+})$ assumed to be 1.14; *cf.* C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc., Ser. A*, **235**, 518 (1956). Invariance of $\phi_{t\to c}$ for stilbene at both 366 and 406 nm confirms the assumption.

(10) S. Wawzonek and E. M. Smolin, *Org. Syn.*, **29**, 83 (1949).

(11) R. A. Caldwell, *J. Amer. Chem. Soc.*, **92**, 1439 (1970).

(12) Stilbene did in fact quench fluorenone fluorescence slightly at higher concentrations in acetone. This effect is expected to be small at the modest (*ca.* 0.06 *M*) concentrations employed in the isomerizations, and we were unable to detect concentration dependence of the isomerization quantum yields.

(13) Analyzed as 95% deuterium (by mass spectral analysis) in the vinyl position (by nmr).

(14) R. A. Caldwell and G. W. Sovocool, *J. Amer. Chem. Soc.*, **90**, 7138 (1968).

(15) R. A. Caldwell and S. P. James, *ibid.*, **91**, 5184 (1969).

(16) W. G. Herkstroeter and G. S. Hammond, *ibid.*, **88**, 4769 (1966).

Since the lifetimes of triplet exciplexes in general, and the one we suggest in particular, are totally unknown, it is not possible to give even an estimate of $k_Q$. It does, however, seem safe to say that the lifetime must be much too short to permit $k_Q$ to be of the order of magnitude of that for $T_1$–$S_0$ radiationless transitions in aromatic hydrocarbons of comparable triplet excitation energy,[17] *ca.* 1 $sec^{-1}$. It thus appears probable that new radiationless decay mechanisms must be considered.

Finally, we wish to point out explicitly that the effect we observe may occasionally complicate determination of $\phi_{ST}$ values by the Hammond–Lamola technique;[2] we would suggest that at least two independent systems be tried when utmost confidence in $\phi_{ST}$ is required.

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(17) W. Siebrand, *J. Chem. Phys.*, 47, 2411 (1967).
(18) G. S. Hammond and D. Valentine, *J. Amer. Chem. Soc.*, in press.

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## Microbial Transformation of Antibiotics. V. Clindamycin Ribonucleotides

*Sir:*

Clindamycin (I) is a clinically useful antibiotic produced by chlorination of lincomycin.[1] Previous papers have described the microbial phosphorylation[2] of clindamycin to clindamycin 3-phosphate (II) and the conversion of clindamycin to *N*-demethylclindamycin or clindamycin sulfoxide by *Streptomyces* species.[3] The present communication describes studies related to the bioconversion of clindamycin to clindamycin ribonucleotides.

Several streptomycete species were found to transform clindamycin to compound(s) lacking *in vitro* antibacterial activity against test organisms. One of these species, *Streptomyces coelicolor*, completely inactivated clindamycin in less than 48 hr when the antibiotic was added to 24-hr cultures of the organism grown in a complex medium. Clindamycin could be regenerated by treatment of the bioinactive fermentation broth with either crude alkaline phosphatase or snake venom phosphodiesterase. This enzymatic behavior suggested that *S. coelicolor* converted clindamycin to compound(s) containing phosphodiester bonds.

(1) B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *Antimicrob. Ag. Chemother.*, 727 (1967).
(2) J. H. Coats and A. D. Argoudelis, manuscript in preparation.
(3) A. D. Argoudelis, J. H. Coats, D. J. Mason, and O. K. Sebek, *J. Antibiot.*, **22**, 309 (1969).

The clindamycin bioconversion products were isolated by adsorption on Amberlite XAD-2 and elution with aqueous methanol. They were purified by chromatography on Dowex-1 (acetate) and counter double current distribution using 1-butanol–water (1:1) as the solvent. Tlc of the obtained material indicated a mixture of bioinactive, uv absorbing compounds which afforded clindamycin by treatment with snake venom phosphodiesterase. Chromatography of the mixture on DEAE-Sephadex (acetate) using Tris–acetate (pH 8.0, 0.1–0.2 *M*) buffer gave eight compounds designated A, B, C, D, E, F, G, and H, in order of elution from the column. Characterization data on compounds A, D, E, G, and H are presented in Table I. Compounds B, C, and F were isolated in small amounts and are not completely characterized.[4]

Compound H was identified as clindamycin 3-phosphate (II) by comparison ($[\alpha]_D$, ir and nmr spectra) with an authentic sample.[2] This material afforded clindamycin by treatment with alkaline phosphatase (Table II) but remained unchanged after incubation with either snake venom or spleen phosphodiesterase.

The molecular formulae of compounds A, D, E, and G (specifically the presence of one P atom per molecule), the uv spectra, and the hydrolysis of these compounds by crude alkaline phosphatase to clindamycin and cytidine, adenosine, uridine, and guanosine (Table II) suggested structures for these compounds in which clindamycin is linked to the phosphate group of cytidine phosphate (compound A), adenosine phosphate (compound D), uridine phosphate (compound E), and guanosine phosphate (compound G). The inability of spleen phosphodiesterase to cleave all four clindamycin ribonucleotides, contrasted with hydrolysis to clindamycin and the corresponding nucleoside 5′-phosphates (Table II) by snake venom phosphodiesterase, indicated a nucleoside 5′-phosphate–clindamycin linkage in these compounds.

We propose structures III, IV, V, and VI for compounds A, D, E, and G, respectively. The assignment of the phosphate diester linkage at the C-3 position of the aminosugar moiety of clindamycin is based on periodate oxidation studies. In this oxidation, it was found that cytidine, adenosine, uridine, guanosine, and their respective 5′-phosphates consumed 1 mol of periodate in less than 15 min with no overoxidation. It was also found that clindamycin rapidly consumed 2 mol of periodate by cleavage of the glycol groupings at C-2, C-3, and C-4 and slowly an additional mol by oxidation of the sulfur. Compounds A, D, E, and G consumed 2 mol of periodate (one rapidly and one slowly) which indicates phosphodiester attachment at C-3 since alternative attachments (C-2 or C-4) would require consumption of 3 mol of periodate.

The results obtained by chemical hydrolyses (Table II) support the postulated structures. As expected,[5] the purine ribonucleotides (compounds D and G) afforded purines and ribose by treatment with 1 *N* aqueous HCl,

(4) Data available at present suggest that compound B is clindamycin sulfoxide 5′-adenylate. The formation of this compound is not surprising since clindamycin is converted to clindamycin sulfoxide by *Streptomyces* species (see ref 3). Compound C appears to be clindamycin 4-(5′-adenylate) and it is most probably produced by rearrangement of the adenyl group under the isolation conditions. Similarly, compound F appears to be clindamycin 4-(5′-guanylate).
(5) E. Chargraff and J. N. Davidson, "The Nucleic Acids," Vol. I, Academic Press, New York, N. Y., 1955, Chapter 5.