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Mechanistic and Exploratory Organic Photochemistry

Organic photochemistry has developed in 15 years into a highly sophisticated field of research.

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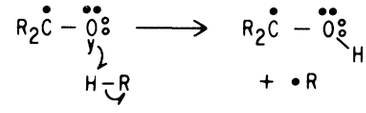
My interest in organic photochemistry began in about 1957. At that time, organic photochemistry was in a relatively primitive stage, approximating that of ground state organic chemistry in 1936. Photochemistry had not reached the point where one could describe in any electronic detail the molecular change occurring during a transformation. Despite thorough and elegant work by physical photochemists, there was a mechanistic discontinuity between the absorption of light and the observation of the primary photochemical products of a reaction. Furthermore, a somewhat indiscriminate nature was ascribed to photochemical reactions, with the reaction being considered a mere consequence of the energy of the wavelength light absorbed. Along the same lines, migration of energy along saturated portions of a molecule, from the absorbing chromophore to the reaction site, was often said to be occurring.

I suggested (*1*, *2*) in 1960 that photochemical reactions are not indiscriminate consequences of energy but are subject to just as severe constraints as are ground state reactions. One of these constraints is the requirement for minimum electron localization. Like ground state molecules, excited states prefer energy valleys and mountain passes to mountain tops. I suggested that, given a resonance or molecular orbital representation of an excited state, one might predict the potential pathways available for reaction. At the time, relatively few excited state structures were available.

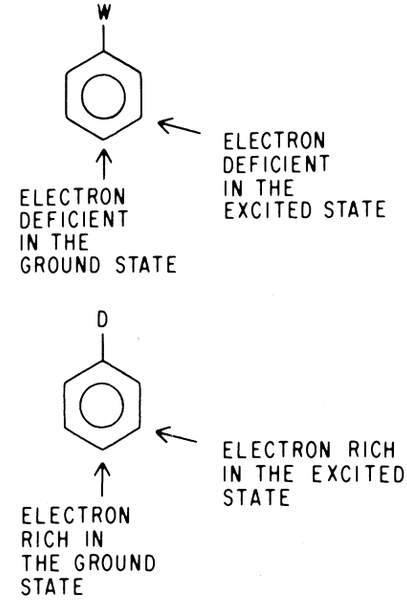
One starting point was the three-dimensional structure of the n-π^* excited state of carbonyl compounds (Fig. 1). This can be written in two dimensions for convenience as shown; and this picture, either three-dimensional or two-dimensional, conveniently serves to allow prediction or understanding of photochemical transformations. For example, the p_y oxygen orbital of the n-π^* excited state is similar to the counterpart orbital of a simple alkoxyl radical; and one would therefore expect easy hydrogen abstraction. This conclusion and the three-dimensional representation were independently arrived at by Kasha (*3*), and it has indeed been demonstrated that one of the characteristics of n-π^* excited states is hydrogen abstraction. Simplifying, we can use two-dimensional shorthand and write a mechanism for hydrogen abstraction from a donor:



My colleagues and I proposed this mechanism along with the comment that this was one of a group of reactions in which excited state of reactant led directly to ground state of product without need for a radiationless transition (*1*). This point has been made subsequently in slightly different notation by Salem (*4*).

The important point is that implicit in this reasoning is the assumption that excited state reactivity can be understood on the same basis as ground state chemistry. Also, we were able at this stage to provide qualitative mechanisms for many kinds of photochemical reactions.

Over the years we have used such qualitative resonance reasoning and also molecular orbital methods with the same philosophy in mind. Our approach has been to search for new photochemical transformations and to see to what extent these are reasonable on the basis of the excited state structure. One interesting example of the utility of this reasoning was our observation that in the excited state we commonly find transmission of electron density meta to and from electron donating and withdrawing substituents (*5*). This contrasts with ground state behavior in aromatic compounds.



These results derived from our molecular orbital calculations. This brings us to the point that molecular orbital reasoning often proves necessary in understanding photochemical reactivity.

In the intervening years we studied photochemical transformations to discern patterns of excited state reactivity and then to correlate these with excited state structure (*6*). During these years we investigated the photochemistry of 4,4-diarylcyclohexe-

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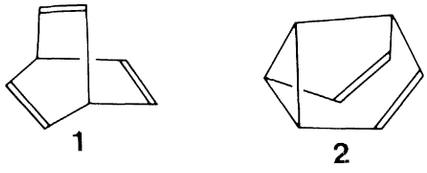
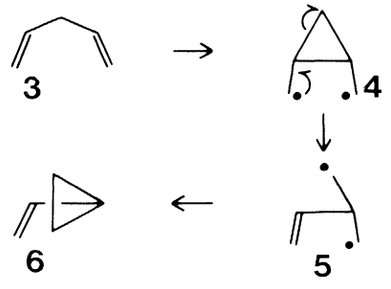
nones (*7*), 4,4-disubstituted cyclohexadienones (*8, 9*), epoxyketones (*10*), acylcyclopropanes (*11*), 4-alkylcyclohexenones (*12*), bicyclo[3.1.0]hex-3-en-2-ones (*13*), bicyclo[3.1.0]hexan-2-ones (*14*), and dibenzoylethylenes (*15*). Each of these comprises a long set of investigations and we focus attention here on a more limited and more recent set of studies.

Thus, in 1966 we reported (*16, 17*) the photochemical rearrangement of barrelene **1** to semibullvalene **2** (Eq. 1). It was quickly recognized (*16, 18, 19*) that the reaction mechanism needed to account for this rearrangement was more broadly general, and we termed the reaction the di-π-methane rearrangement.

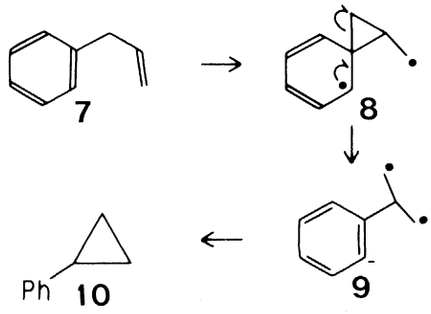


EQUATION 1

We noted that in general a di-π-methane rearrangement requires two π-moieties bound to a single, sp^3 hybridized (that is, "methane") carbon. Barrelene, of course, has several such groupings of moieties. All the rearrangements can be accounted for by one basic mechanism which may be expressed in simple resonance terms as in Eq. 2a. Here two vinyl groups are used for simplicity to depict the mechanism. However, in Eq. 2b phenyl groups may replace the ethylenic bond.



EQUATION 2a



EQUATION 2b

The product then is either a vinylcyclopropane or an arylcyclopropane. If one of the double bonds is a carbonyl group, this then is the oxa-di-π-methane rearrangement (*20*). As has been noted (*21*) the di-π-methane rearrangement has developed into one of the most general of photochemical reactions. It seems to follow a number of consistent mechanistic patterns so that it is possible to predict the reaction course with modest certainty in any given example. As a consequence, the reaction also promises to be of considerable utility in syntheses.

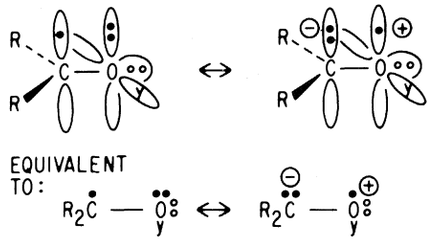


Fig. 1. The n-π^* excited state of carbonyl compounds.

Among the generalizations, the following can be briefly stated: (i) In a divinylmethane system that is unsymmetrically substituted, it is the π-bond with the least number of phenyl groups that survives the reaction (*22*). (ii) The stereochemistry at the vinyl group of product is the same as in reactant (*23*). (iii) The configuration at the methane carbon (that is, C-3) is inverted (*24*). (iv) The reaction is stereospecific at the double bond terminus, becoming part of the three-membered ring, with the *cis* reactant giving the *cis* product and the *trans* reactant becoming the *trans* product (*25*). (v) Substitution on the methane carbon facilitates the reaction. Without substitution, such as geminal methyl, the reaction does not proceed (*26*). (vi) Bicyclic di-π-methane systems (barrelene, for example) tend to rearrange via their triplets, while acyclic di-π-methanes tend to utilize their singlet states (*19*). The multiplicity correlation is better tied to presence or absence of a nonconstrained double bond (a potential "free rotor"), which tends to dissipate excitation faster than to react (*27*).

In our studies of the di-π-methane rearrangement we initially used reaction quantum yields (that is, millimoles of product per milliEinstein of light absorbed) as a measure of reactivity. However, quantum yields are not ideal measures of inherent molecular reactivity since they are merely probabilities of an excited state reacting rather than doing other things, such as decaying to the ground state. What is really desired is the rate at which an excited state reacts to give photoproduct.

In the case of the di-π-methane rearrangement of acyclic systems, the rearranging singlet has an exceptionally short lifetime, one ranging from a nanosecond to less than a picosecond (that is, 10^{-9} to 10^{-12} second). Measurements of rates of disappearance of such short-lived species posed a special challenge.

If we can measure the lifetime of an excited singlet and also its quantum yield of reaction, we can derive very simply the unimolecular rate at which it rearranges.

$$\phi_r = k_r/({}^1k_{dt}) = k_r\,\tau \qquad (3a)$$

or

$$k_r = \phi_r({}^1k_{dt}) = \phi_r/\tau \qquad (3b)$$

Thus, in Eqs. 3a and 3b, ϕ_r is the quantum yield for the reaction, ${}^1k_{dt}$ is the total unimolecular rate of decay of the excited singlet (including all processes such as rearrangement, decay in the absence of radiation, intersystem crossing to give a triplet, and fluorescence), and τ is the lifetime of the excited singlet and the reciprocal of ${}^1k_{dt}$. Using these equations and knowing two of three unknowns such as ϕ_r and ${}^1k_{dt}$ or ϕ_r and τ, we can solve for the desired rate k_r. Now, ϕ_r is routinely measured experimentally and we are left only with our need for ${}^1k_{dt}$ or its reciprocal τ.

One method of obtaining short lifetimes of excited singlets is single photon counting (*28*). In this method a flash lamp of duration 0.5 to 4 nanoseconds, a multichannel analyzer, and sensitive photomultiplier are used, along with some additional instrumentation. Fluorescence emitted by the sample after each lamp flash is attenuated so that at most only one photon emitted is captured by the photomultiplier detector; and after each flash, we determine the time delay before photon ejection by the sample. The multichannel analyzer is used to record the number of single photons emitted at each time delay, and the number at each delay is proportional to the excited singlet concentration at that time. Thus we obtain excited state concentration as a function of time.

However, for lamp flashes that have durations comparable to the lifetime of an excited state under study, the excited state population is being augmented throughout the duration of the lamp flash and thus throughout the period of excited state decay. This means that the function giving excited state concentration versus time does not correspond to a simple negative exponential and does not directly afford the unimolecular rate constant.

In principle, knowing the lamp intensity as a function of time and also knowing the fluorescence intensity emitted as a function of time, we can dissect the unimolecular decay function in a process known as "deconvolution." This process has been noted (*29*) to be unstable mathematically and to afford unreliable results.

One approach is deconvolution by convolution, that is, assuming a decay function and then seeing the extent to which the

observed lamp flash, together with this function, predicts the observed fluorescence emission (*28, 29*).

In recent investigations of our own, we described a method wherein this reiterative convolution is done systematically, and especially rapid convergence is obtained (*30*). When this method was used, along with an interfaced minicomputer to fulfill the role of the multichannel analyzer and also to carry out the calculations on-line, we were able to measure lifetimes as short as 100 picoseconds with an error of ±14 picoseconds (*30, 31*).

Nevertheless, the method still proved capable of dealing with only a portion of the excited singlet reactions of interest, since many of the di-π-methane rearrangements had rates of rearrangement and decay which corresponded to even shorter lifetimes (*30*). Then it was found that at low temperatures the lifetimes of the di-π-methane reactant molecules increased and the rates of decay (that is, the $^1k_{dt}$'s) decreased, often by 200-fold in proceeding from room temperature to 77°K. Thus, molecules with lifetimes too short to measure at room temperature commonly had measureable lifetimes at the temperature of liquid nitrogen, 77°K (*30*). The experimental observation was an increased intensity of fluorescence at low temperature. Thus, in Eq. 4

$$\phi_f^{77}/\phi_f^{RT} = \frac{k_f/(^1k_{dt}^{77})}{k_f/(^1k_{dt}^{RT})} = (^1k_{dt}^{RT})/(^1k_{dt}^{77}) = M \quad (4)$$

where RT refers to room temperature and M the ratio of fluorescence quantum yields at the two temperatures. Absolute quantum yields need not be obtained, but rather, only relative fluorescence intensities at two temperatures, 77°K and room temperature. In Eq. 4, we have assumed that k_f, the natural rate of fluorescence emission (that is, the rate in absence of any competing processes) is independent of temperature; this is a fair assumption for molecules with relatively undistorted excited states, and we have been able to check this point experimentally (*30*).

Hence, being able to obtain M experimentally, we can solve Eq. 4 for a room temperature decay rate which is too rapid to measure directly and obtain this rate in terms of the low temperature rate which is slower and measurable. This is seen in Eq. 5.

$$^1k_{dt}^{RT} = M\,^1k_{dt}^{77} \quad (5)$$

Using this approach we were able to determine the reaction and decay rates for a number of singlet di-π-methane rearrangements. Some of these are summarized in Fig. 2.

Inspection of Fig. 2 reveals that the fastest rate measured is the excited singlet decay of 1,1,5,5-tetraphenyl-3,3-dimethyl-1,4-pentadiene (**17**) where $^1k_{dt}^{RT} = 1.8 \times 10^{12}$ sec^{-1}, corresponding to a lifetime of 0.55 picosecond. While such short-lived species can be studied by laser techniques, the single photon counting technique has as an advantage that it is easy to use, and each measurement is relatively routine.

We note from Fig. 2 that the rates of rearrangement tend to increase as terminal substitution by stabilizing groups is increased. Thus, tetraphenyldiene **17** rearranges more rapidly than diphenyldimethyldiene **13** by a factor of about 20, and this rearranges about 11-fold more rapidly than the diene **11**, which has just two terminal phenyl groups and no terminal methyl groups. Thus, phenyl substitution is more effective than methyl which, in turn, is more helpful than hydrogens. It is also clear that phenyl substitution at other positions, such as C-2, is not effective in increasing the reaction rate. Furthermore, while replacement of the central (that is, the methane carbon) methyl groups by phenyl groups (compare compound **13** with **15**) does not appreciably affect the rearrangement rate, the absence of such central methyl substitution dramatically inhibits rearrangement (see **19**). This pattern of reactivity makes sense if bridging between the two vinyl groups is rate-limiting in the excited singlet as depicted in Fig. 3.

One other point of interest is in Fig. 2. This bears on the point made earlier that quantum yields really do not give accurate

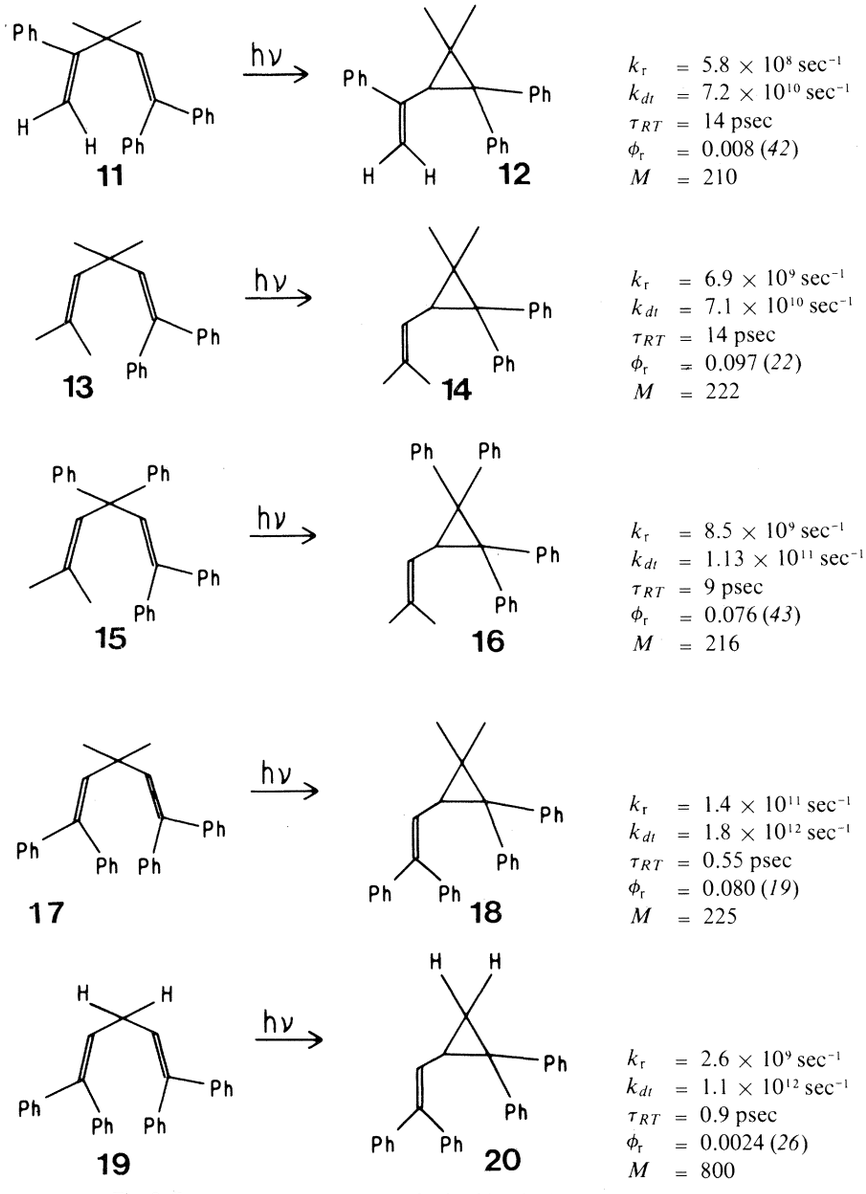


Fig. 2. Some rearrangement rates obtained by single photon counting (*30*).

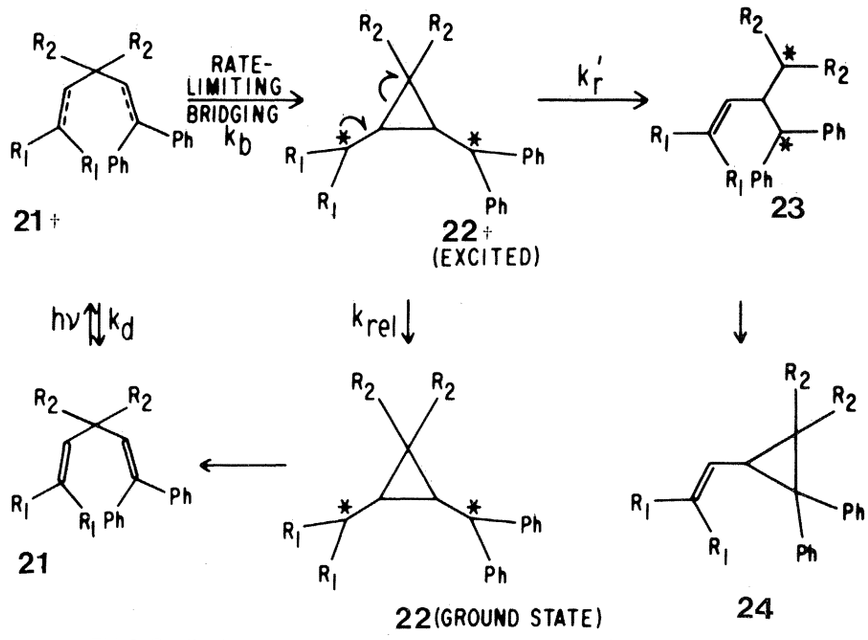


Fig. 3. Qualitative valence bond mechanism for the di-π-methane rearrangement.

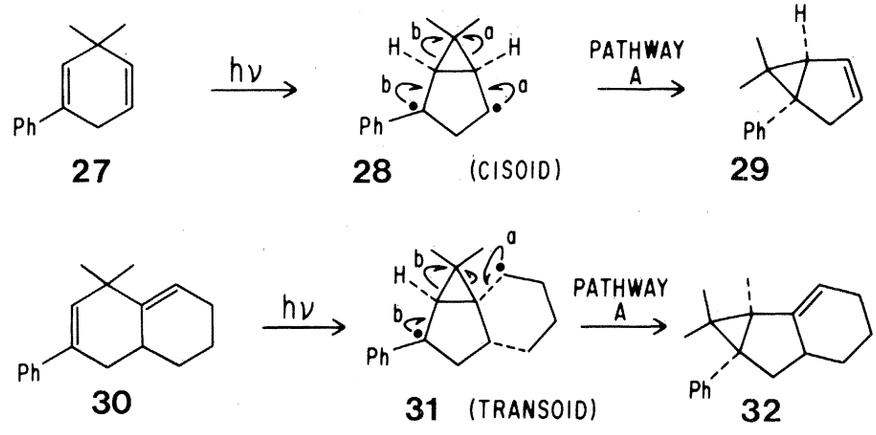


Fig. 4. Generation of cisoid and transoid diradicals in the di-π-methane rearrangement.

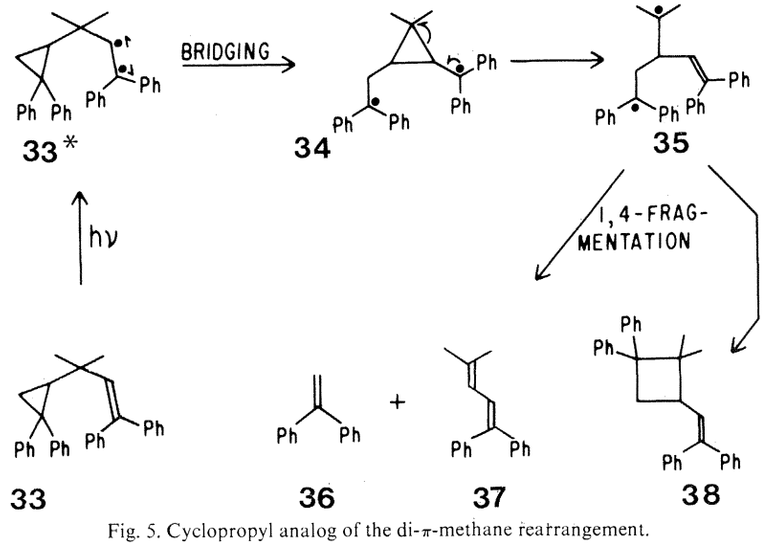
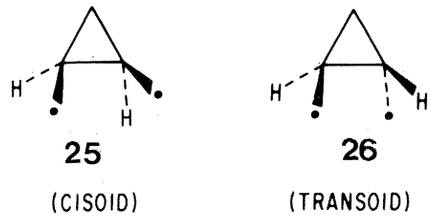


Fig. 5. Cyclopropyl analog of the di-π-methane rearrangement.

measures of excited state reactivity. Thus, in comparing di-π-methane systems **13** and **17**, we find that the quantum yields are really quite similar (that is, $\phi_r = 0.097$ and $\phi_r = 0.080$, respectively), this despite the fact that compound **13** has only two terminal phenyl groups compared with four for compound **17**. Inspection of the rates of rearrangement, however, reveals the 20-fold enhancement in rate for compound **17** noted above.

One other facet of the di-π-methane rearrangement is of some interest and is stereochemical. This is the question whether the cyclopropyldicarbinyl diradical species utilized in the rearrangement are necessarily of one stereochemistry, cisoid or transoid (that is, as **25** or **26**).



This question was answered in a study (*32*) in which the two di-π-methane systems **27** and **30** were used. It was observed that both of the rearrangements do occur. Since di-π-methane reactant **27** necessarily proceeds via cisoid diradical **28** and di-π-methane reactant **30** uses the transoid diradical **31**, it is clear that both cyclopropyldicarbinyl diradicals may be successfully utilized in the di-π-methane rearrangement (Fig. 4).

One interesting variation of the di-π-methane rearrangement replaces one of the two π-bonds with a three-membered ring (*33*). The details are outlined in Fig. 5. The products obtained derive from a process quite analogous to that of the ordinary di-π-methane rearrangement. However, consideration of the quantitative aspects of the reaction indicates that the process of vinyl-cyclopropyl excited state bonding is less favorable than the usual vinyl-vinyl bridging. Thus, the quantum yield for the reaction is only $\phi = 0.018$; and the rate, as determined by single photon counting, is only $k_r = 8.7 \times 10^8$ sec^{-1} (compared with **17** in Fig. 2, where $k_r = 1.4 \times 10^{11}$ sec^{-1}).

Turning now away from di-π-methane photochemistry, we recently (*34*) synthesized a series of dioxetanes (**39, a–d**) which, on four-membered ring fission, can yield 4,4-diphenylcyclohexadienone (**40**). It was known from the work of Kopecky and Mumford (*35*) that thermal cleavage of simple dioxetanes affords triplet excited states of the resulting carbonyl compounds. Also, it was demonstrated by White *et al.* (*36*) that carbonyl compounds

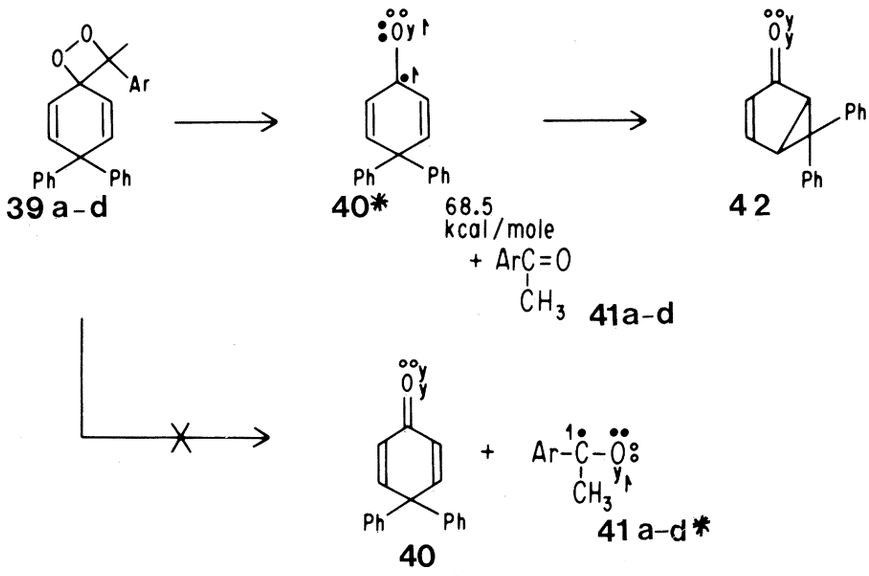


Fig. 6. Dioxetane fission to give intramolecular rearrangement. Triplet energies: dienone, 68.5 kcal/mole; ketone **41a** (Ar = CH_3), 82 kcal/mole; ketone **41b** (Ar = Ph), 74 kcal/mole; ketone **41c** (Ar = *m*-MeOPh), 72 kcal/mole; ketone **41d** (Ar = 2-naphthyl), 59 kcal/mole.

so generated can be used as triplet sensitizers to effect photochemical reactions; here we have intermolecular delivery of triplet excitation. Very recently, Darling and Foote (*37*) and Richardson *et al.* (*38*) described examples in which the dioxetane moiety was incorporated in the reacting molecule and where any energy transfer must thus be intramolecular; fragmentation reactions resulted.

Our recent studies (*34*) differ in that the intramolecular excitation leads to an intramolecular rearrangement. This is the type A rearrangement we had studied many years ago (*8, 9*). A particularly intriguing aspect of this effort is the finding that the reaction efficiency is independent of the triplet energy of the ketone by-product released along with the triplet of 4,4-diphenylcyclohexadienone (Fig. 6). Especially in the case of release of 2-acetonaphthone, which has a triplet energy of only 59 kcal/mole, one might have expected this ketone to compete for the triplet excitation rather than allowing the higher energy (68.5 kcal/mole) 4,4-diphenylcyclohexadienone triplet to be generated.

The solution to this apparent paradox is that the transition state for dioxetane fission has a kinetic preference for formation of n-π^* excited states rather than π-π^* species as has already been noted by Kearns (*39*) and others (*40*). That the n-π^* excited state of 2-acetonaphthone should be of considerably higher energy is suggested by configuration interaction calculations (*41*). Thus, the dioxetane transition state leads directly to the lowest energy n-π^* excited state available and there is no intervention of the low energy 2-acetonaphthone π-π^* triplet. This is not totally surprising since the type A dienone rearrangement is known to be one of the most rapid of triplet rearrangements (*8*).

Most important, though, lack of equilibration of states in the process means that it is indeed the n-π^* excited triplet of the dienone which is the rearranging species; and, this is a question involving some controversy in the past.

In summary, we might consider the present situation in organic photochemistry and its future. Despite the increasing number of known photochemical reactions, the total number of well-established photochemical transformations still is infinitesimal compared with that in ground state chemistry. Thus, there is a field of synthesis through photochemistry which has just barely been born.

Further, our understanding of the factors which control photochemical reactions is still quite primitive. In some instances it appears to be the facility of conversion to ground state which controls the reaction while in other cases it appears to be the energy hypersurface surrounding the excited state, with the pathway with minimum barriers being preferred. In addition, quantum mechanical methods now available for description of excited states are unwieldy and not too practical. Both configuration interaction and inclusion of the three-dimensional sigma framework are desirable; yet this leads to cumbersome wave functions that are not simply interpreted. More complex calculations are not needed; rather, a new approach is needed.

Finally, totally new methods of determining photochemical reaction mechanisms are needed; the number is really quite small when compared with those developed for use in ground state organic chemistry.

Thus, organic photochemistry is just emerging as a major field in its own right. Those who feel that photochemistry, and sometimes even chemistry, is reaching an end, should recognize that progress in a field often is limited by intellectual impasses rather than the nature of the field. Intellectual breakthroughs come only occasionally and only as a consequence of considerable effort, thought, and optimism.

References and Notes

1. *Abstracts*, 17th National Organic Symposium, Bloomington, Ind., June, 1961, p. 31.
2. NSF Proposal, *Mechanistic and Exploratory Organic Photochemistry* (1959); H. E. Zimmerman and D. I. Schuster, *J. Am. Chem. Soc.* **83**, 4486 (1961); *ibid.* **84**, 4527 (1962).
3. M. Kasha, *Radiation Res. Suppl.* **2**, 243 (1960); in *Light and Life*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1961), p. 31.
4. L. Salem, *J. Am. Chem. Soc.* **96**, 3486 (1974); ———, W. G. Dauben, N. J. Turro, *J. Chim. Phys. Phys.-Chim. Biol.* **70**, 694 (1973).
5. H. E. Zimmerman and V. R. Sandel, *J. Am. Chem. Soc.* **85**, 915 (1963); H. E. Zimmerman and S. Somasekhara, *ibid.*, p. 922; H. E. Zimmerman, *Adv. Photochem.* **1**, 183 (1963).
6. H. E. Zimmerman, *Science* **153**, 837 (1966).
7. ———, and J. W. Wilson, *J. Am. Chem. Soc.* **86**, 4036 (1964); H. E. Zimmerman and K. G. Hancock, *ibid.* **90**, 3749 (1968); H. E. Zimmerman and N. Lewin, *ibid.* **91**, 879 (1969); H. E. Zimmerman and W. R. Elser, *ibid.*, p. 887; H. E. Zimmerman and R. L. Morse, *ibid.* **90**, 954 (1968); H. E. Zimmerman, R. D. Rieke, J. R. Scheffer, *ibid.* **89**, 2033 (1967); H. E. Zimmerman, R. C. Hahn, H. Morrison, M. C. Wani, *ibid.* **88**, 159 (1966).
8. H. E. Zimmerman and J. S. Swenton, *ibid.* **86**, 1436 (1964); *ibid.* **89**, 906 (1967).
9. H. E. Zimmerman and G. Jones, *ibid.* **91**, 5678 (1969).
10. H. E. Zimmerman and R. D. Simkin; *Tetrahedron Lett.* **28**, 1847 (1964); H. E. Zimmerman, H. G. Dürr, R. G. Lewis, S. Bram, *J. Am. Chem. Soc.* **84**, 4149 (1962); H. E. Zimmerman, B. R. Cowley, C.-Y. Tseng, J. W. Wilson, *ibid.* **86**, 947 (1964).
11. H. E. Zimmerman, S. S. Hixson, E. F. McBride, *J. Am. Chem. Soc.* **92**, 2000 (1970); H. E. Zimmerman and C. M. Moore, *ibid.*, p. 2023; H. E. Zimmerman and T. W. Flechtner, *ibid.*, p. 6931.
12. H. E. Zimmerman, R. G. Lewis, J. J. McCullough, A. Padwa, S. Staley, M. Semmelhack, *ibid.* **88**, 159 and 1965 (1966); H. E. Zimmerman and D. J. Sam, *ibid.*, pp. 4114 and 4905.
13. H. E. Zimmerman, R. Keese, J. Nasielski, J. S. Swenton, *ibid.*, p. 4895; H. E. Zimmerman and J. O. Grunewald, *ibid.* **89**, 3354 and 5163 (1967); H. E. Zimmerman, D. Döpp, P. S. Huyffer, *ibid.* **88**, 5352 (1966); H. E. Zimmerman and D. S. Crumrine, *ibid.* **90**, 5612 (1968); ———, D. Döpp, P. S. Huyffer, *ibid.* **91**, 434 (1969).
14. H. E. Zimmerman, K. G. Hancock, G. Licke, *ibid.* **90**, 4892 (1968).
15. H. E. Zimmerman, H. G. Dürr, R. S. Givens, R. G. Lewis, *ibid.* **89**, 1863 (1967); H. E. Zimmerman, R. S. Givens, R. M. Pagni, *ibid.* **90**, 4191 (1968); H. E. Zimmerman and J. Hull, *ibid.* **92**, 6515 (1970).
16. H. E. Zimmerman and G. Grunewald, *ibid.* **88**, 183 (1966).
17. H. E. Zimmerman, R. W. Binkley, R. S. Givens, G. L. Grunewald, M. A. Sherwin, *ibid.* **91**, 3316 (1969).
18. H. E. Zimmerman, R. W. Binkley, R. S. Givens, M. A. Sherwin, *ibid.* **89**, 3932 (1967).
19. H. E. Zimmerman and P. S. Mariano, *ibid.* **91**, 1718 (1969).
20. R. S. Givens and W. F. Oettle, *Chem. Commun.* (1969), p. 1164; W. G. Dauben, M. S. Kellogg, J. I. Seeman, W. A. Spitzer, *J. Am. Chem. Soc.* **92**, 1786 (1970).
21. S. S. Hixson, P. S. Mariano, H. E. Zimmerman, *Chem. Rev.* **73**, 531 (1973).
22. H. E. Zimmerman and A. C. Pratt, *J. Am. Chem. Soc.*, **92**, 1407 and 6259 (1970).
23. ———, *ibid.*, pp. 1409 and 6267.

24. H. E. Zimmerman, J. D. Robbins, R. D. McKelvey, C. J. Samuel, L. R. Sousa, *ibid.* **96**, 1974 and 4630 (1974).
25. H. E. Zimmerman, P. Baeckstrom, T. Johnson, D. W. Kurtz, *ibid.* **94**, 5504 (1972); *ibid.* **96**, 1459 (1974).
26. H. E. Zimmerman and J. A. Pincock, *ibid.* **94**, 6208 (1972); *ibid.* **95**, 2957 (1973).
27. H. E. Zimmerman, K. S. Kamm, D. P. Werthemann, *ibid.* **96**, 7821 (1974); *ibid.* **97**, 3718 (1975); H. E. Zimmerman and G. A. Epling, *ibid.* **92**, 1411 (1970); *ibid.* **94**, 8749 (1972); H. E. Zimmerman and G. E. Samuelson, *ibid.* **89**, 5971 (1967); *ibid.* **91**, 5307 (1969).
28. W. R. Ware, in *Creation and Detection of the Excited State*, A. A. Lamola, Ed. (Dekker, New York, 1971), vol. 1, part A; L. M. Bollinger and G. E. Thomas, *Rev. Sci. Instrum.* **32**, 1044 (1961); G. Laustriat, A. Choche, H. Lami, G. Pfeffer, *C. R. Acad. Sci.* **257**, 434 (1963); W. R. Ware, L. J. Doemeny, T. L. Nemzek, *J. Phys. Chem.* **77**, 2038 (1973).
29. A. E. W. Knight and B. K. Selinger, *Spectrochim. Acta Part A* **27**, 1223 (1971).
30. H. E. Zimmerman, D. P. Werthemann, K. S. Kamm, *J. Am. Chem. Soc.* **95**, 5094 (1973); *ibid.* **96**, 439 (1974).
31. H. E. Zimmerman and T. P. Cutler, *Chem. Commun.* (1975), p. 598.
32. H. E. Zimmerman and L. M. Tolbert, *J. Am. Chem. Soc.* **97**, 5497 (1975).
33. H. E. Zimmerman and C. J. Samuel, *ibid.*, pp. 448 and 4025.
34. H. E. Zimmerman and G. E. Keck, *ibid.*, p. 3527.
35. K. R. Kopecky and C. Mumford, *Can. J. Chem.* **47**, 709 (1972).
36. E. White, J. Wiecko, D. R. Roswell, *J. Am. Chem. Soc.* **92**, 2167 (1970).
37. T. R. Darling and C. S. Foote, *ibid.* **96**, 1625 (1974).
38. W. H. Richardson, F. C. Montgomery, M. B. Yelvington, *ibid.* **94**, 9277 (1974).
39. D. R. Kearns, *Chem. Rev.* **71**, 345 (1971).
40. E. M. Evleth and G. Feler, *Chem. Phys. Lett.* **22**, 499 (1973); D. R. Roberts, *J. Chem. Soc. Chem. Commun.* (1974), p. (→ J. J. S. Dewar and S. Kirschner, *J. Am. Chem. Soc.* **96**, 7578 (1974); N. J. Turro and A. Devaquet, *ibid.* **97**, 3859 (1975).
41. H. E. Zimmerman, R. W. Binkley, J. J. McCullough, G. A. Zimmerman, *J. Am. Chem. Soc.* **89**, 6589 (1967).
42. H. E. Zimmerman and A. A. Baum, *ibid.* **93**, 3646 (1971).
43. H. E. Zimmerman, R. J. Boettcher, W. Braig, *ibid.* **95**, 2155 (1973).
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Evolution of Repeated DNA Sequences by Unequal Crossover

DNA whose sequence is not maintained by selection will develop periodicities as a result of random crossover.

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A considerable portion of the DNA of some eukaryotes consists of sequences repeated very large numbers of times (*1*). These highly repetitious DNA's are often called satellites. The repeated unit is relatively homogeneous within each species, but major differences are observed between related repetitious DNA's in different species, even of the same genus (*2, 3*). Some satellites have been shown to consist of short, relatively homogeneous tandem repeats; the repeats in different satellites ranged in length from 2 to about 12 base pairs (*2, 4*). Other repetitious DNA's are more complex. For instance, partial sequence analysis of guinea pig alpha satellite (*5*) and mouse satellite (*6*) shows that neither is composed of a single very short repeating sequence. Instead, these DNA's appear to contain subrepeats of homologous but not identical sequences within a larger repeating unit.

Botchan (*7*) and Southern (*8*), among others, have investigated long-range periodicities in the more complex repetitious DNA's by digesting them with restriction enzymes, which cleave DNA at particular base pair sequences. This approach is illustrated by the work of Southern (*8*) on digestion of mouse satellite DNA with the restriction enzyme Eco RII. The major products are fragments whose lengths are small integral multiples of about 240 base pairs. Thus there seems to be an approximately 240 base pair periodicity in this DNA, with some of the repeats missing the Eco RII site, so that some fragments of higher multiples of 240 base pairs are released. In addition to these major fragments, small amounts of "fractional" fragments with lengths equal to 0.5, 1.5, 2.5, . . . times 240 base pairs are also obtained. The 120 base pair and 360 base pair fractional fragments are released in roughly equimolar amounts. Southern points out that these equimolar yields make it very unlikely that the fractional fragments arise exclusively by straightforward mutation somewhere near the middle of the 240 base pair repeat to produce new Eco RII sites; for in that case most such mutations would result in two 120 base pair restriction fragments, and very few would result in 360 base pair fragments. I will discuss the origin of fractional fragments later. When purified 240 base pair fragments were denatured and allowed to reassociate, a large proportion of the reassociated DNA was in high molecular weight complexes formed by reassociation of the complementary strands in a staggered register. This shows that the 240 base pair unit is composed of subrepeats, thus confirming the indirect conclusion from sequence analysis (*6*).

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Role of Unequal Crossover

I will argue in this article that repetitious DNA's with these characteristics will arise and evolve naturally as a result of random unequal crossover between sister chromatids—that is, between the two daughters produced by replication of a single DNA molecule. These unequal crossovers, which must occur in the germ line to be evolutionarily significant, might happen either at meiosis or at any one of the many germ line mitoses.

Repetitious DNA's might arise and evolve by many different mechanisms. I have singled out unequal crossover because there is good evidence that it actually occurs. Sister chromatid crossovers, which might be either equal or unequal, have been demonstrated to occur at a rate of several exchanges per cell per division in a variety of eukaryotic cells (*9, 10*). In many of these studies, exchange was detected with the aid of bromodeoxyuridine or [^{3}H]thymidine, which can artificially induce crossovers. Nevertheless it is very likely that there is an appreciable rate of crossover even in the absence of artificial induction, since exchanges occur at roughly comparable rates in ring chromosomes, where they can be detected without artificial agents by virtue of producing dicentric rings (*10*). I know of no direct evidence for unequal sister chromatid crossover. However, unequal nonsister chromatid crossover has been well known since the work of Bridges and Sturtevant on the *bar* locus of *Drosophila* (*11*), and there is indirect evidence for unequal sister chromatid exchange at the *bar* (*12*) and ribosomal RNA (*bobbed*) (*13*) loci of the same organism. I think that this evidence, taken together, strongly suggests that unequal sis-