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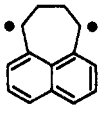
Biradicaloid Intermediates in Photochemistry: Spectroscopic and Kinetic Study of 1,4-Perinaphthadiyl and Related 1,8-Naphthoquinodimethanes

Sir:

Information regarding the electronic structure, energy, and reactivity of biradicaloid intermediates in their ground and low-lying excited states is scarce but essential for a mechanistic understanding of most photochemical[1] and many thermal reactions. We present such information for 1,4-perinaphthadiyl (**1**), a π,π biradical generated by a light-induced nitrogen elimination and studied by ESR and time-resolved absorption and emission spectroscopy in the range of 4–350 K. While first attempts to detect the optical spectrum of parent 1,8-naphthoquinodimethane (**2**) have failed,[2] the triplet state of both **1**[3] and **2**[4] has been identified by ESR spectroscopy, and Michl et al.[5] have recently reported optical and ESR spectra of 1,3-perinaphthadiyl (**3**). Our present results indicate that previous[3,5] determinations of the singlet–triplet energy gap of **1** and **3** are erroneous and that the absorption and emission spectra attributed to singlet **3**[5] should be reassigned.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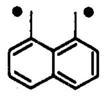
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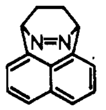
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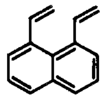
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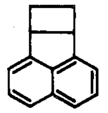
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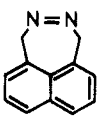
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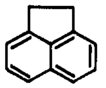
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Prolonged irradiation of the azo compound **4**[3] in rigid glasses at 77 K results in a quantitative conversion into 1,8-divinylnaphthalene (**5**). Analysis of the progressive changes in the absorption spectrum of a 10^{-4} M solution by the method of Mauser[6] reveals that the conversion is biphotonic, proceeding via an intermediate which is stable in the dark at 77 K. Upon monochromatic excitation at 405 nm the intermediate accumulates to an extent of up to 60%. Its absorption spectrum was obtained by deducing contributions from **4** and/or **5** according to the ratios given by the analysis[6] for various stages of the conversion. The intermediate behaves as a single, uniform species: its highly structured absorption spectrum is faithfully reproduced by the excitation spectrum of its intense fluorescence ($\tau = 190 \pm 10$ ns at 77 K) at $\lambda > 512$ nm (Figure 1). The formation of the species and its conversion into **5** upon further irradiation or to 6b,7,8,8a-tetrahydrocyclobut[*a*]acenaphthylene (**6**) upon warming above 100 K is paralleled by the buildup and decay of the characteristic ESR signals attributed[3] to the triplet state of **1**. Both ESR and optical spectra are strikingly similar to those of **3**,[5] prepared by a different photoreaction. Furthermore, a similar absorption (weak band system at $\lambda \leq 507$ nm, strong bands at $\lambda \leq 337$ nm), attributed to **2**, slowly builds up upon 365-nm irradiation of the azo compound **7** at 77 K. Apparently only a small fraction of the photochemical conversion of **7** into acenaphthene (**8**) proceeds via the stable intermediate **2** and we have not been able to accumulate sufficient quantities for a proper determination of its entire UV-vis absorption spectrum. In addition, **2** is very sensitive to light and practically nonluminescent.

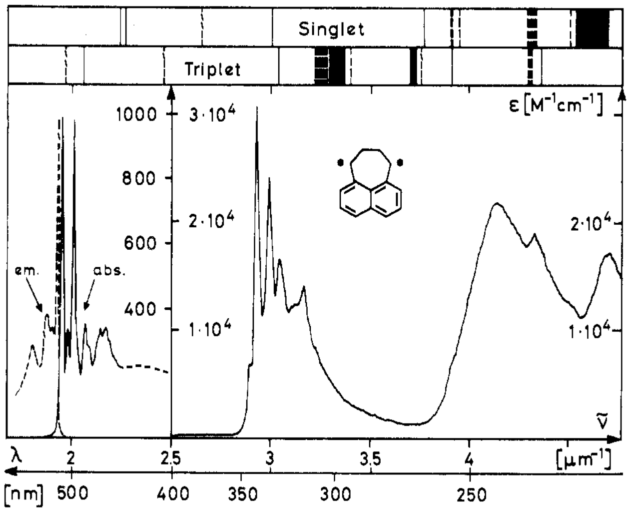

Figure 1. Absorption and emission spectrum of **1** in EPA (ether:isopentane:alcohol, 5:2:2) at 77 K. Top: electronic transitions predicted by PPP calculations for planar **2** in its lowest singlet and triplet state. Transition energies are indicated by vertical bars (full lines, transition moment parallel; broken lines, orthogonal to C_2 axis); thickness reflects calculated oscillator strength. For the singlet manifold an additional, long-wavelength transition is predicted at 0.87 μm^{-1} (forbidden, orthogonal).

What is the multiplicity of the electronic states giving rise to the optical spectra of **1** to **3**? From ESR signal intensity variations with temperature (90–130 K for **1** and **3**, 12.5–29 K for **2**), it has been concluded that the three biradicals have a singlet ground state, the energy gap to the lowest triplet state amounting to 200 (**1**),[3] 45 (**2**),[4] and 640 (**3**)[5] cal/mol. We deduce that at 77 K the singlet/triplet ratios should be 1.3 (**1**), 0.5 (**2**), and 21 (**3**). The close similarity between the three absorption spectra is thus rather surprising, particularly since the S–S and the T–T transitions predicted by open-shell PPP SCF CI calculations[7] differ considerably (top of Figure 1) and only the triplet calculation is in satisfactory[8] agreement with experiment. Clearly inconsistent with the ESR results for **1** was the finding that both shape and intensity of its absorption were quite insensitive to a variation of the sample temperature in the range of 30–130 K. Hence, we investigated the ESR signal intensity dependence of **1** and **3** in the temperature range from 77 down to 4 K, where the thermal population of an excited triplet state should become negligible. A DPPH standard was used to check for instrumental artefacts. Relative intensities obtained from the peak to peak heights or by double integration of the derivative curves were comparable. Amazingly, the signal intensities of both **1** and **3** were nearly[9] proportional to $1/T$, as predicted by Curie's law for molecules with a triplet ground state. We may conclude that the triplet state is the electronic ground state of **1** and **3**, or lies within a few calories/mole above the lowest singlet state. If the deviations[3,5]

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from Curie's law above 77 K are real,[10] they can not be interpreted in terms of a two-level scheme, but require more elaborate models such as site dependence of the energy gap or different heat capacities for different states. At any rate, we attribute the optical spectra of **1** and **3** observed at 77 K to transitions within the triplet manifold, although a minor contribution from S–S transitions in the absorption spectrum cannot be excluded. This assertion might be directly proven by MCD spectroscopy and is supported by the following observations.

In spite of the near coincidence of the 0-0 absorption and emission bands of **1** at 512 nm, there is an apparent lack of mirror symmetry between the two spectra, suggesting that the absorption band is composed of more than one electronic transition. The PPP calculation indeed predicts two weak transitions of opposite polarization in that region (509 and 486 nm). These have been resolved by the method of photoselection. Irradiation of a randomly oriented sample of **1** in PMMA at 77 K with linearly polarized light ($\lambda > 510$ nm) produced a sample of remaining **1** which exhibited pronounced linear dichroism. The spectrum was then analyzed by the method of Albrecht[11] to yield two orthogonal components with 0-0 vibronic bands at 510 and 496 nm, respectively. The component corresponding to the $T_0 \rightarrow T_1$ transition is an approximate mirror image of the emission spectrum. The quantum yield of fluorescence at 77 K is estimated as 0.3 from the radiative rate constant of 1.4×10^6 s^{-1}, obtained by integration of the T_0–T_1 absorption band, and the experimental lifetime. It is raised to near unity upon cooling to 20 K.

Room temperature flash photolysis of **4** in various solvents reveals **1** as a transient intermediate formed within 100 ns after excitation and with a lifetime of 200 ± 20 μs. The same transient is observed after sensitized excitation of **4** in the presence of benzophenone. If high concentrations of **1** are produced by very intense flashes, the decay is at first dominated by a second-order process (T–T annihilation). An Arrhenius plot for the first-order decay rate of **1** in glycerol as a function of temperature (25 to 75 °C) has yielded the parameters $A = 10^{6.9 \pm 0.3}$ s^{-1}, $E_a = 4.5 \pm 0.3$ kcal/mol (s.e., six points). The low value of the preexponential factor (cf.[5] $A(\mathbf{3}) = 10^{4.5 \pm 1}$ s^{-1}) suggests that the rate-determining step involves a spin-forbidden process, namely, intersystem crossing to S_1 which rapidly decays to products. Indeed, the lifetime of **1** is sensitive to heavy-atom effects, being reduced to 20 μs in methylene bromide and 30 μs in a solution of 0.2 M dimethylmercury in methanol at room temperature. Neither the yield nor the lifetime of **1** are appreciably affected by the addition of piperylene or maleic anhydride in concentrations up to 0.5 M. However, oxygen and nitric oxide efficiently quench the transient absorption of **1** with bimolecular rate constants of $(1.3 \pm 0.3) \times 10^{10}$ and $(2.5 \pm 0.5) \times 10^{10}$ M^{-1}s^{-1}, respectively, in methanol. Both oxygen and nitric oxide are partly consumed by the quenching reaction. Under continuous irradiation at room temperature, the conversion $\mathbf{4} \rightarrow \mathbf{5} + \mathbf{6}$ is, of course, monophotonic, since the lifetime of the intermediate **1** is too short to permit the absorption of a second photon. The profound effects of some of the above-mentioned substrates on the product distribution[12] fully substantiate the results from the direct observation of **1** by flash photolysis.

Some conclusions and some of the unresolved questions are discussed in the following. The naphthoquinodimethanes **1** to **3** have been observed in a bound electronic state of triplet multiplicity which is stable in rigid glasses below 80 K. Light-induced nitrogen elimination from **4** largely or exclusively yields triplet **1** as a primary photoproduct both in solution at room temperature (flash photolysis and trapping experiments[12]) and in rigid glasses at 77 K. Triplet **1** has a lifetime of ~200 μs in solution at room temperature. Its reactivity toward dienes or maleic anhydride is low, and these substrates are inefficient as trapping agents. This agrees with the observations on related triplet diyls such as trimethylenemethane and its derivatives[13] and the biradicaloid triplet intermediates in photoenolization[14] and Norrish type II reactions[15] of aromatic ketones.

Little direct information is available on the properties of the lowest singlet state of **1** to **3** and it is not certain whether the biradicaloid geometry corresponds to a local minimum on the lowest singlet surface of the free molecules. Both the absence of distinct features attributable to S–S transitions in the absorption spectra and the activation energies of 4.5 kcal/mol for the decay of **1** and **3**,[5] which probably reflect the energy needed for the T–S intersystem crossing, suggest that the triplet state of **1** and **3** is the ground state. Note, however, that the quenching rate of **1** by oxygen is close to one half the diffusion controlled limit and is thus equal to the maximum rate expected from spin statistics.[16] Hence, every encounter complex of overall triplet multiplicity leads to $^3O_2$ and singlet ground-state products. The changes in product distribution[12] resulting from oxygen quenching of triplet **1** are not exceptional[14,15,17] and may simply reflect a difference in the geometry preferred for the intersystem crossing in the presence and absence of oxygen, respectively.

The ESR intensity studies leave a number of open questions. The low-temperature data clearly show that the previous results obtained in the range of 90–130 K are either artificial or have been misinterpreted. Careful studies will be needed to decide between these two alternatives as well as to substantiate or disprove the small deviations from Curie's law observed with **1**[9] and **2**[4] at very low temperatures where saturation effects and errors in the sample temperature are difficult to avoid. In any case, S–T splittings determined from ESR intensity measurements in a narrow temperature range must be considered with caution.[18]

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(8) The (gratifying) numerical agreement of the triplet calculation with the first observed weak and strong transitions should not be overvalued and may well be somewhat fortuitous. However, we feel that two discrepancies between the singlet calculation and the experiments cannot be ignored. (i) Both Michl's[5] and our calculations predict the first S–S transition near 1100 nm. In spite of careful searches no absorption of **1** to **3** was detected beyond 520 nm. (ii) All calculated S–S transitions down to 230 nm are forbidden, or only weakly allowed. The observed bands near 330 nm are very strong.

(9) The signal intensities of **1** in both hexafluorobenzene and poly(methyl methacrylate) were somewhat less than predicted from Curie's law at temperatures below 20 K. Standard analysis[3-5] would locate the triplet state at ~20 cal/mol above the singlet ground state. However, in view of the inconsistencies encountered we are reluctant to attribute any significance to this value.

(10) A redetermination of the ESR signal intensities with samples of **1** in the range of 90–130 K has yielded similar data to those obtained earlier.[3] However, the apparent deviations from Curie's law were within a conservative estimate of the possible systematic errors. A reexamination of **3** is underway in Professor Michl's laboratories (personal communication).

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Rapid Scanning Stopped-Flow Absorption Studies of the Effect on Tryptophanase of a Change in pH or K$^+$ Concentration: Evidence for a Slow Conformational Change[1]

Sir:

Tryptophanase, a pyridoxal-P-dependent enzyme requiring monovalent cations for optimum catalytic activity[2-4] has absorption maxima at 337 and 420 nm which have been attributed[4] to active and inactive forms, respectively. The amplitudes of these interconvertible absorptions depend upon pH and the type and concentrations of monovalent cations.[5] We now report that their interconversion following a change in pH or K$^+$ concentration occurs on the stopped-flow time scale. This new result indicates the occurrence of either enzyme conformational changes in the interconversion process or slow protonation–deprotonation steps. Such slow changes have important implications for the mechanism of catalytic action of a large class of pyridoxal-P-dependent enzymes. For example, the amplitudes and rates of biphasic formation of an enzyme bound quinonoid observed with tyrosine phenol lyase,[6] serine hydroxymethylase,[7] and tryptophanase[8] vary with pH[7,8] and monovalent cations.[8] It is likely that the slow changes described here contribute to this kinetic complexity.

Holotryptophanase from *Escherichia coli* B/1t 7-A, prepared as described previously,[9,10] was studied at 24 °C in three experiments:[11] (A) a pH drop from 8.53 to 6.72; (B) a pH jump from 7.38 to 9.30; (C) a sudden change (K$^+$ jump) from 0.1 M Na$^+$ to 0.05 M Na$^+$ and 0.05 M K$^+$ at pH 8.0.

A computerized double-beam rapid-scanning absorbance stopped-flow system[12] was used to collect 150 spectra per second over the wavelength range 280–550 nm. The abrupt spectral changes observed in these experiments occurred within the 6.5-ms dead time (1.85-cm-path-length cell), while the slower changes required seconds to minutes. The absorbance–wavelength–time data as well as spectra from control experiments (enzyme spectra, the change in the pyridoxal-P spectrum with pH[13] and background spectra) were collected with a PDP 8/I computer. The software permits substraction of the contribution of free pyridoxal-P, determination of the enzyme absorbance, $A_0(\lambda)$ (the expected spectrum after mixing in the absence of change), and construction of difference spectra.

Most of the effects of pH can be rationalized by Scheme I which assumes rapid protonation and deprotonation (involving one or more protons) of two slowly interconvertible enzyme forms. The high pH form, E′, has an absorption peak at 337 nm (Figure 1, ○), while the low pH form, EH$^+$, has its absorption maximum at 420 nm (Figure 1, ▲). After a pH drop, E′ forms E′H$^+$ to give an abrupt decrease in absorbance at ~330 nm ($\Delta A = -0.075$) and a corresponding increase at 390 nm ($\Delta A = +0.033$) (Figure 1, ●). These changes are followed by first-order decay at ~340 nm ($\Delta A = -0.054$) and simultaneous growth at ~420 nm ($\Delta A = +0.071$) with $k_2 = 0.43 \pm 0.03$ s^{-1}. This slow change is indicative of a conformational change from E′H$^+$ to EH$^+$.

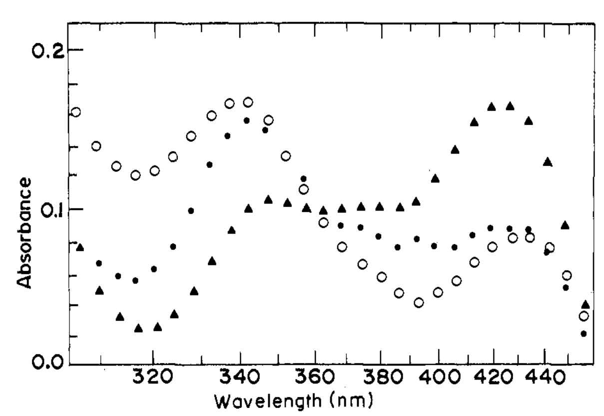

Figure 1. Tryptophanase spectra (path length 1.85 cm) corrected for free pyridoxal-P absorption, observed in the pH drop experiment: zero time, ○; first spectrum after mixing (t = 6–12 ms), ●; spectrum after completion of the first-order process (t = 8 s), ▲.

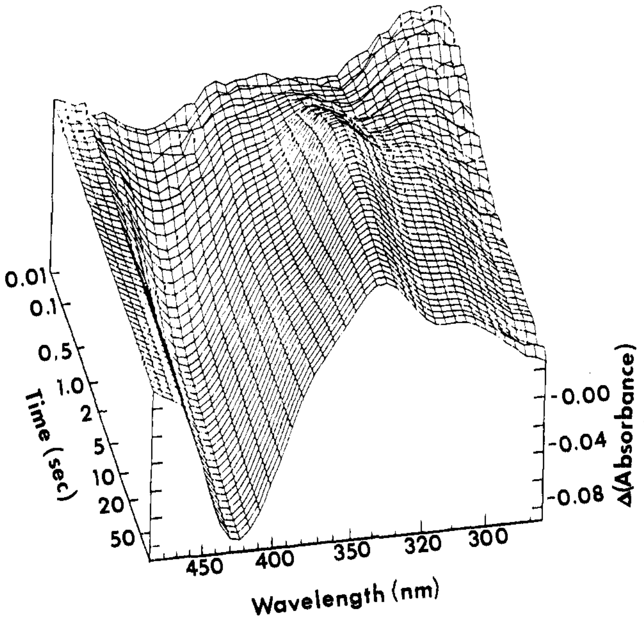

Figure 2. Absorbance difference–wavelength–time surface observed in the pH jump experiment. The spectra of free pyridoxal-P and of tryptophanase at zero time ($A_0(\lambda)$) have been substracted to give these difference spectra.

The overall changes for the pH jump experiment are the reverse of those observed for the pH drop, but the time sequence and the intermediates involved are different. Figure 2 is a view of the three-dimensional surface which shows the time evolution of the difference spectrum, $A(\lambda) - A_0(\lambda)$. After the pH jump, EH$^+$ is converted to E yielding an abrupt absorbance increase at ~300 nm ($\Delta A = 0.056$) and a decrease at 420 nm ($\Delta A = -0.047$). Then a first-order growth at ~340 nm ($\Delta A = +0.106$) and simultaneous decay at ~420 nm ($\Delta A = -0.133$) occur as E is converted to E′ with $k_1 = 0.56 \pm 0.03$ s^{-1}. This is followed by a slower first-order decay of absorbance

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