part is concerned, they are more similar to each other than any two thymine or uracil containing structures of which I am aware."[18] This finding strongly supports our suggestion[31,32] that isologous sulfur and selenium compounds should be isosteric to such an extent that differences in biological activity should be ascribable not to differences in their ability to fit receptor sites, but rather to differences in electron distribution, thus adding further interest to the synthesis and biological study of sulfur and selenium isologs related to biologically active compounds.

The results reported here are in agreement with the prediction[10] that bond length and bond order of carbon–sulfur bonds in pyrimidines should be position dependent. It was also shown that the bond lengths of sulfur–hydrogen bonds were position dependent. It would be tempting to extrapolate from these data to the differences in chemical reactivity and biological activity of positional isomers of thiopyrimidines already noted; however, such speculations would seen to be premature in view of the lack of knowledge about the mechanisms involved.

(31) W. H. H. Gunther and H. G. Mautner, *J. Med. Chem.*, 7, 229 (1964).
(32) W. H. H. Gunther and H. G. Mautner, *J. Am. Chem. Soc.*, 87, 2708 (1965).

Electron Paramagnetic Resonance Studies of the Role of Solvent in Chlorophyll-Photosensitized One-Electron-Transfer Reactions Involving Quinones and Hydroquinones[1]

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Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721. *Received October 7, 1966*

Abstract: Chlorophyll-photosensitized one-electron-transfer reactions involving quinones and hydroquinones have been carried out in a variety of solvents. Two principal conclusions have emerged: (a) solvent electrons are not involved in semiquinone radical formation; (b) carbonyl-containing solvents form complexes with the semiquinone radicals causing marked changes in spin density distributions and thus in the hyperfine structure of the epr spectrum. Experiments using β-carotene as a quencher demonstrate that the chlorophyll triplet state is involved in both oxidation and in reduction processes.

We have been interested in understanding the mechanism of porphyrin-photosensitized, one-electron-transfer reactions as a means of obtaining insight into the role of chlorophyll in photosynthesis.[2-5] Our previous work has demonstrated the reversible formation of semiquinone free radicals upon illumination of degassed ethanol solutions of porphyrins and quinones or hydroquinones. It was proposed that radical formation is the result of a single electron transfer between electronically excited porphyrin and an oxidant or reductant. The present paper is a result of attempts to assess the role of the solvent in these reactions. This work has provided us with further insight into the mechanism of semiquinone formation in these systems.

Experimental Section

Electron paramagnetic resonance (epr) spectra were obtained with a Varian 100-kc modulation spectrometer, equipped with a V-FR2200 Fieldial unit and a TE_{102} cavity. A Leeds and Northrup Speedomax G recorder was used to monitor the output of the spectrometer.

For irradiation involving porphyrin excitation, a 500-w tungsten filament projection lamp was focused through a water bath containing an infrared-absorbing heat filter and through a Corning-No. 3-66 filter (which passes only wavelengths longer than 5500 A) into the cavity of the epr spectrometer.

For irradiation involving quinone excitation, a high-pressure mercury arc (Osram HBO-100w/2) was focused into the epr spectrometer cavity by means of two quartz condensing lens.

Chlorophyll *a* was prepared by the method of Anderson[6] from spinach leaves. Pheophytin (*a* and *b*) and hematoporphyrin were obtained from Fluka AG and protoporphyrin IX was obtained from Calbiochem. All were used without further purification. *p*-Benzoquinone and 1,4-naphthoquinone were obtained from Eastman and purified by sublimation. Hydroquinone and 1,4-naphthalenediol (Eastman) were recrystallized from water. Propionaldehyde, cyclohexanone, aceptophenone, and dimethylacetamide were redistilled before use. Acetic acid (Du Pont, reagent grade), ethanol (U.S.I., absolute), monochloroacetic acid (Mallinckrodt AR), dichloroacetic acid (Matheson Coleman and Bell, Reagent Grade), trichloroacetic acid (Eastman), benzoic acid (Mallinckrodt AR), formic acid (Mallinckrodt, 97–100%), and potassium acetate (Mallinckrodt AR) were used without further purification.

Results and Discussion

In Figure 1a is shown a typical epr spectrum obtained by illuminating a degassed solution of chlorophyll *a* and *p*-benzoquinone in ethanol with red light.[2] The spectrum is that characteristic of the *p*-benzosemiquinone free radical as observed, for example, by chemical reduction of *p*-benzoquinone in aqueous base. The

(1) This work was supported in part by the U. S. Atomic Energy Commission, Contract No. AT(11-1)908, and by the U. S. Air Force Cambridge Research Laboratories, Contract No. 19(628)4376.
(2) G. Tollin and G. Green, *Biochim. Biophys. Acta*, **60**, 524 (1962).
(3) G. Tollin and G. Green, *ibid.*, **66**, 308 (1963).
(4) G. Tollin, K. K. Chatterjee, and G. Green, *Photochem. Photobiol.*, **4**, 593 (1965).
(5) A. K. Banerjee and G. Tollin, *ibid.*, **5**, 315 (1966).
(6) A. Anderson, Bio-Organic Chemistry Quarterly Reports, University of California Radiation Laboratory (UCRL-10951), Aug 1963.

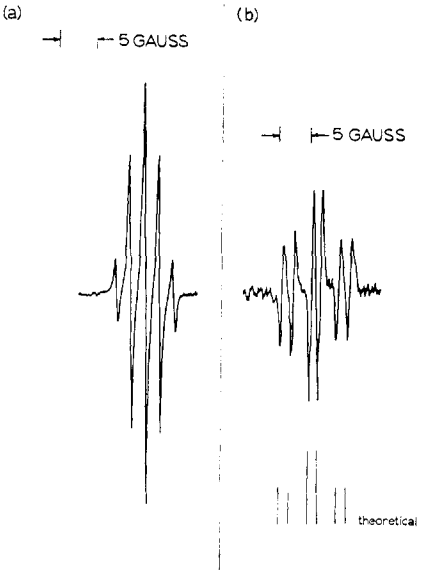


Figure 1. Epr spectra obtained upon illumination with red light of a degassed solution of chlorophyll *a* (2×10^{-5} *M*) and *p*-benzoquinone (10^{-3} *M*): a, ethanol; b, glacial acetic acid.

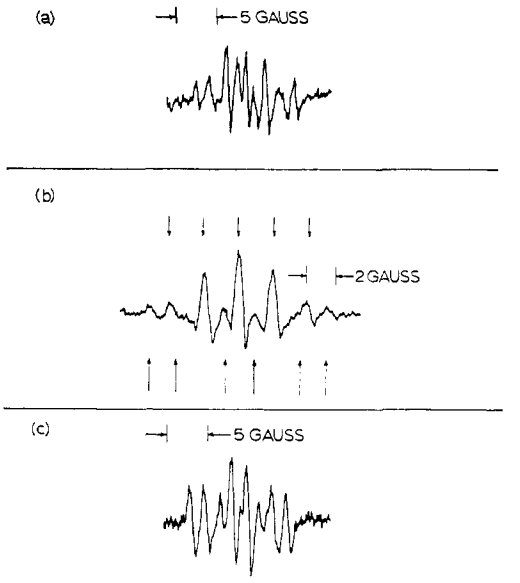
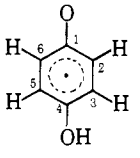


Figure 2. Epr spectra obtained upon irradiation with red light of a degassed solution of pheophytin (*a* and *b*) (2×10^{-5} *M*) and *p*-benzoquinone (10^{-3} *M*): a, 1:3 acetophenone–ethanol; b, 1:3 dimethylacetamide–ethanol; c, 1:3 propionaldehyde–ethanol.

spectral shape is the result of a hyperfine coupling between the unpaired electron and four equivalent protons on the aromatic ring.[7] In Figure 1b is shown the spectrum obtained when the solvent is glacial acetic acid.[9] As is evident, this spectrum is quite different from that obtained in ethanol. Its interpretation requires a hyperfine interaction between the unpaired electron and three protons, two of which have equal, large coupling constants and the third of which has a smaller coupling constant (Table I). Yamazaki and

Table I. Hyperfine Coupling Constants for Benzosemiquinone Radical in Glacial Acetic Acid[a]



α_{H_3}	= 5.0 gauss
α_{H_5}	= 5.0 gauss
$\alpha_{H(-OH)}$	= 1.9 gauss

[a] See text for justification of assignments.

Piette[8] have observed the epr spectrum of the undissociated *p*-benzosemiquinone radical in aqueous acid using continuous-flow methods. Three lines, with an intensity ratio of 1:4.4:1, were obtained. This was interpreted in terms of two proton splittings, with exchange reactions causing the deviation from the expected intensity ratio of 1:2:1. As is evident, these results are quite different from those obtained in the present study. However, it is significant that the protonation, by making the two oxygens nonequivalent, changes the spin density distribution in the radical (see below).

Several possibilities exist which can account for the appearance of a new spectrum when acetic acid is used in place of ethanol: (a) the semiquinone radical is replaced by a solvent-derived radical, (b) the semiquinone radical is replaced by a porphyrin-derived radical, and (c) a solvent-induced modification of the semiquinone radical is occurring. The following experiments were performed to test these possibilities.

Various combinations of ethanol and acetic acid (ranging from 1:1 to 3:1) were found to give results similar to those obtained with glacial acetic acid. When compounds related in structure to acetic acid were used, the results shown in Figure 2 were obtained.[10] In addition, six-line signals identical with that shown in Figure 1b were obtained using the following compounds in ethanol solution: mono-, di-, and trichloroacetic acids, benzoic acid, and formic acid.

A careful examination of the spectra in Figures 1 and 2 reveals that the more complex spectra in Figure 2 can be interpreted in terms of a superposition of the "normal" five-line benzosemiquinone spectrum and the six-line "acetic acid" spectrum. This is shown quite clearly in Figure 2b in which arrows are used to mark the expected line positions.

A definitive demonstration of this interpretation is given by experiments in which the ratio of propionaldehyde to ethanol is varied. These are shown in Figure 3. Thus, as the proportion of propionaldehyde is decreased, the six-line spectrum decreases in relative intensity and the five-line spectrum becomes dominant. This is quite clear evidence that we are dealing with two separate species whose relative concentrations are dependent upon the solvent composition. Further,

(7) This implies that the two oxygens in the semiquinone are equivalent, which would be the case for the anion radical. Yamazaki and Piette[8] have estimated the p*K* for the benzosemiquinone radical as 4.25. Thus, one might expect to have the anionic species in a solvent such as ethanol (see ref 8 for the various benzosemiquinone structures).

(8) I. Yamazaki and L. H. Piette, *J. Am. Chem. Soc.*, 87, 986 (1965).

(9) The characteristics of this system are quite similar to those described earlier[2,4] for the ethanol system, *i.e.*, light absorbed by chlorophyll is involved in radical production, the electron-transfer process is completely reversible, and the reaction is essentially temperature independent. Similar results are obtained with pheophytin.

(10) Because of the fact that carbonyl compounds detune the epr apparatus, it was often not possible to do experiments with pure solvents.

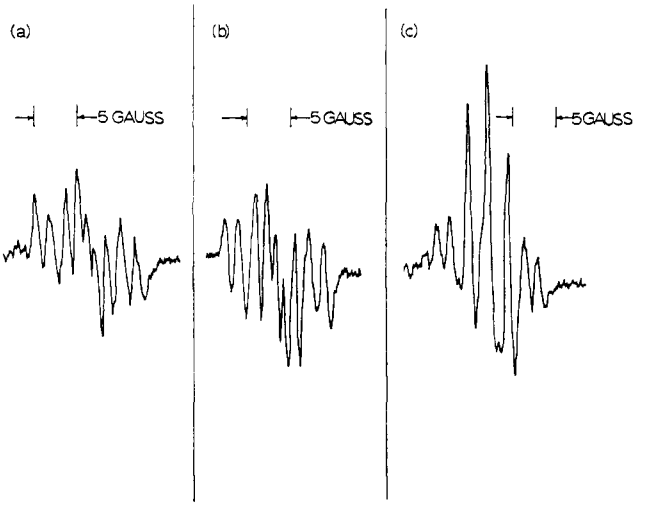


Figure 3. Epr spectra obtained upon irradiation with red light of a degassed solution of pheophytin (*a* and *b*) (2×10^{-5} *M*) and *p*-benzoquinone (10^{-3} *M*): a, 5:20 propionaldehyde–ethanol; b, 3:22 propionaldehyde–ethanol; c, 1:24 propionaldehyde–ethanol.

the fact that such a wide variety of solvents give rise to the same radical species proves that the radical cannot be solvent derived.

Changing the porphyrin from chlorophyll to pheophytin to hematoporphyrin to protoporphyrin IX has no effect on the signal shape, although signal intensities are markedly different. This suggests, but does not prove, that the new radical species is not porphyrin derived.[11] Further evidence on this point was obtained by experiments in which semiquinone radical species were produced either chemically or photochemically in systems not containing porphyrins. For example, powdered zinc added to acetic acid solutions of *p*-benzoquinone gave small, but easily recognizable, six-line spectra. Similarly, ultraviolet irradiation of acetic acid solutions of *p*-benzoquinone gave identical results (Figure 4). When the ultraviolet irradiation was carried out in pure ethanol, a typical five-line benzosemiquinone spectrum was obtained.

The above results strongly suggest that the six-line epr spectrum is due to a modified form of the semiquinone radical. In order to test this further, we performed experiments with different quinones. The spectra observed with 1,4-naphthoquinone in glacial acetic acid and in ethanolic KOH are shown in Figure 5. The spectrum in the ethanol–KOH is the normal one for the naphthosemiquinone free radical (a similar, although smaller, signal is obtained upon illumination of porphyrin–quinone solutions in ethanol or acetic acid saturated with potassium acetate). The line shape obtained in acetic acid is quite different and is interpretable in terms of a hyperfine coupling involving four protons, three with equivalent, but small, coupling constants and one with a much larger constant (Table II). *p*-Chloranil gives only a single line spectrum in either ethanol or acetic acid. It is thus quite clear that the epr spectra under all conditions are the result of radical species derived from the quinones and not from either solvent or porphyrin.

(11) It is conceivable that the radical could have been associated with the cyclopentanone ring system of chlorophyll, with a side chain attached to the macrocyclic ring or with the central pyrrole nitrogen system.

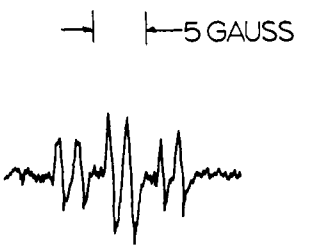


Figure 4. Epr spectrum obtained upon ultraviolet irradiation of a solution of *p*-benzoquinone (0.1 *M*) in 3:7 acetic acid–ethanol.

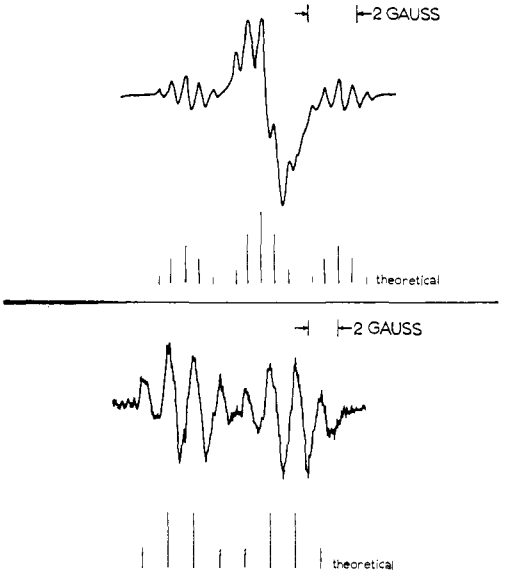
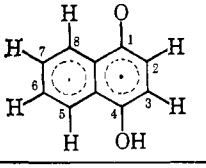


Figure 5. Epr spectra obtained with solutions of 1,4-naphthoquinone (10^{-3} *M*): a, ethanol–KOH (no porphyrin, dark); b, glacial acetic acid (pheophytin present; degassed and irradiated with red light).

Still further support for the above concept comes from experiments we have carried out with the corresponding diols (hydroquinone and 1,4-naphthalenediol). These give results which are identical with those obtained with the quinones.

Table II. Hyperfine Coupling Constants for Naphthosemiquinone Radical in Glacial Acetic Acid[a]



α_{H_8}	= 6.8 gauss
$\alpha_{H(-OH)}$	= 1.7 gauss
α_{H_5}	= 1.7 gauss
α_{H_7}	= 1.7 gauss

[a] See text for justification of assignments.

Inspection of effectiveness of various types of compounds in producing the modified epr spectra reveals the following rough sequence of activity: carboxylic acids > aldehydes, ketones, amides > esters (actually, esters give only the "normal" epr spectra). In agreement with this, we find that phosphoric acid is quite effective in producing the modified signal (*e.g.*, 5 μl

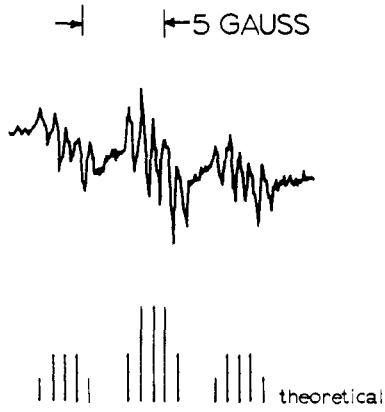


Figure 6. Epr spectrum obtained upon irradiation with red light of a degassed solution of pheophytin (*a* and *b*) (10^{-4} *M*) and *p*-benzoquinone (10^{-3} *M*) in benzene saturated with benzophenone. A Mnemotron CAT computer was used to obtain this spectrum; 360 scans (3-hr illumination period) were used.

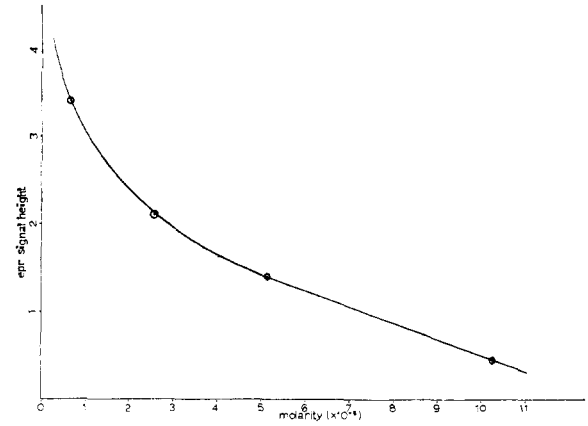
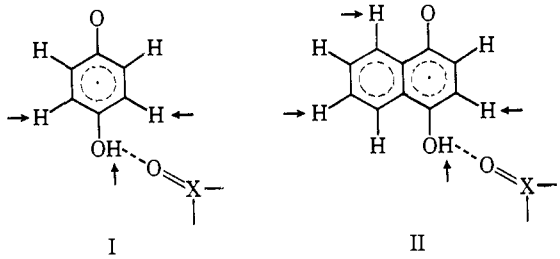


Figure 7. Epr signal height *vs.* β-carotene concentration for the chlorophyll *a*–*p*-benzoquinone system in ethanol.

of phosphoric acid in 25 ml of ethanol gives only a six-line spectrum with *p*-benzoquinone).

If we assume that the carbonyl group in these solvents (or the phosphoryl group in phosphoric acid) is forming a hydrogen bond with the semiquinone free radical[12] which suppresses the ionization of the hydroxyl hydrogen, it is possible to completely rationalize all of the above results. Thus, the structures can be written as



I II

The observed spectra can now be interpreted in terms of interactions with the hydrogens marked by arrows. It would seem reasonable to assign the small coupling (Table I) to the hydroxyl hydrogen and the two larger couplings to the ring hydrogens in the case of benzoquinone. For the naphthoquinone, we can tentatively assign the couplings as in Table II, although alternative schemes are certainly possible. The change from the normal pattern of interaction can be thought of as being caused by the hydrogen bonding which makes the two oxygens in the radical nonequivalent and changes the degree of conjugation between the π orbitals in the ring and the p orbital occupied by the unpaired electron, in analogy with the protonated semiquinone.[8]

It is to be noted that we have included a coupling involving the hydroxyl hydrogen. Ordinarily, couplings of this kind are seldom seen in epr, presumably owing to either rapid rotation of the OH group or to exchange of protons with the medium.[13] Hydrogen bonding, of the type considered here, would be expected to reduce

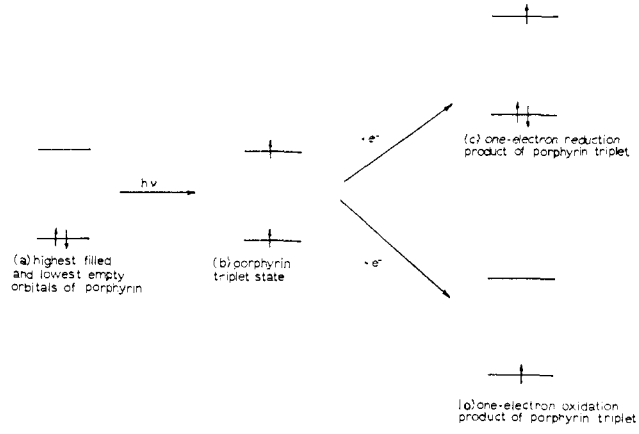


Figure 8. Schematic electron orbital diagram showing one-electron oxidation and reduction of the porphyrin triplet state.

both of these effects, thus allowing the hyperfine interaction to be observed.

Further evidence in support of this hypothesis is provided by experiments in which potassium acetate is added to glacial acetic acid. Under these conditions, only the "normal" epr spectra are obtained. This can be rationalized in terms of removal of the hydroxyl hydrogen in the radical by the basic acetate ion which thus prevents hydrogen bonding and allows the anion radical to be formed.

A further variety of epr spectrum is observed in solvents such as ether[14] and benzene[15] (saturated with benzophenone). This is shown in Figure 6. This spectral pattern can be constructed by the addition of two small proton couplings to the basic six-line spectrum (Table III). Perhaps an intermediate strength hydrogen bond is formed in these solvents, leading to a partial delocalization of spin density to include the two other protons of the benzene ring system.

The fact that so many chemically different solvent systems will permit the formation of quinone radicals supports the idea[2] that solvent electrons are not involved in the photochemical process. Thus, the porphyrin itself must be the only source of redox electrons.

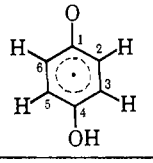
(12) We are ignoring hydrogen bonds formed between the radical and ethanol, although these undoubtedly occur. Most likely, the fact that two radical species occur simultaneously in mixed solvent systems is due to competition between the solvents in hydrogen bond formation.

(13) D. J. E. Ingram, "Free Radicals as Studied by Electron Spin Resonance," Butterworth and Co., London, 1958, p 174.

(14) A slow photobleaching of the chlorophyll occurs in this solvent, probably as a result of side reactions. Also, ethanol–ether mixtures give only the "normal" five-line spectrum.

(15) No epr signals are observed in pure benzene, or in pure hydrocarbons generally.

Table III. Hyperfine Coupling Constants for Benzosemiquinone Radical in Ethers[a]



α_{H_3}	= 5.5 gauss
α_{H_5}	= 5.5 gauss
$\alpha_{H(-OH)}$	= 1.5 gauss
α_{H_2}	= 0.75 gauss
α_{H_6}	= 0.75 gauss

[a] See text for justification of assignments.

Inasmuch as porphyrins can function either as one-electron reductants[2] or as one-electron oxidants,[5,16,17] it is important to ask whether the same excited state is involved in both processes. Fujimori and Tavla[17] have shown that β-carotene, a specific quencher of the chlorophyll triplet, is capable of preventing the chlorophyll-sensitized photoxidation of hydroquinone. We have confirmed this and have also shown that β-carotene can quench the chlorophyll-sensitized reduction of *p*-benzoquinone (Figure 7). From these data, one can calculate a Stern–Volmer quenching constant of $4 \times 10^4\ M^{-1}$. From the data of Fujimori and Tavla,[17] a quenching constant of $2.5 \times 10^4\ M^{-1}$ can be calculated. These are in satisfactory agreement, considering the difficulties inherent in the measurements. We can thus conclude that the chlorophyll triplet can indeed function in both types of redox processes. One can visualize this in terms of the diagram in Figure 8. Perhaps this has significance for the functioning of chlorophyll in the two photochemical systems of photosynthesis.[18]

(16) E. Fujimori and M. Tavla, *Nature*, 208, 78 (1965).
(17) E. Fujimori and M. Tavla, private communication.

(18) NOTE ADDED IN PROOF. Recently, Gough (*Trans. Faraday Soc.*, 62, 2321 (1966)) has observed the epr spectrum of the monoprotonated *p*-benzosemiquinone species, produced by ultraviolet irradiation, in a variety of organic solvents. An 18-line spectrum was observed, probably analogous to the spectrum shown in Figure 6 of the present work, although better resolved. The coupling constants are quite similar to those given in Table III above. In addition, evidence for hydrogen bonding of the hydroxyl hydrogen to the solvent is presented.

Communications to the Editor

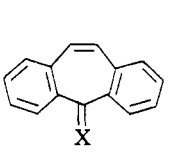
The Stereospecific Addition of Dibenzo[*a*,*d*]cycloheptenylidene and Tribenzo[*a*,*c*,*e*]cycloheptenylidene to Olefins

Sir:

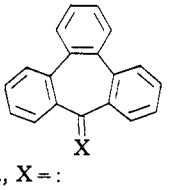
It is known that carbenes substituted by aryl groups add to olefins in a nonstereospecific manner; this has been attributed to the triplet nature of the intermediate involved.[1-3] Thus Skell's hypothesis for the stereochemical course in addition reactions seems still to be valid in spite of the recent criticisms cited in the literatures.[4,5]

We wish to report that aryl-substituted carbenes, *i.e.*, dibenzo[*a*,*d*]cycloheptenylidene (**1a**) and tribenzo[*a*,*c*,*e*]-cycloheptenylidene (**2a**), add to *cis*- and *trans*-2-butenes stereospecifically.[6] The ground-state triplet nature of **1a** and **2a** is clearly shown by electron spin resonance studies.[7]

p-Toluenesulfonylhydrazones **1c**, mp 204° dec, and **2c**, mp 213° dec, are obtained from the corresponding ketones **1b**[8] and **2b**,[9] respectively. The reaction of these



1a, X = :
b, X = O
c, X = NNHSO$_2$C$_6$H$_4$CH$_3$
d, X = N$_2$



2a, X = :
b, X = O
c, X = NNHSO$_2$C$_6$H$_4$CH$_3$
d, X = N$_2$

hydrazones with sodium methoxide in dry pyridine affords the diazo compounds 5-diazodibenzo[*a*,*d*]cycloheptene (**1d**), mp 62° dec (25% yield), and 9-diazotribenzo[*a*,*c*,*e*]cycloheptene (**2d**), mp ~60° dec (4% yield).

The diazo compounds **1d** and **2d** were irradiated in *cis*- and *trans*-2-butenes at 0° with a 1000-w high-pressure mercury lamp.[10] Stereospecific addition of **1a** was confirmed on the following basis. No *trans*-2,3-dimethylcyclopropane derivative was detected during the photolysis of **1a** in *cis*-2-butene. The hydrocarbon fraction obtained was subjected to alumina chromatography and divided into *ca.* 50 fractions. Each fraction was checked by infrared spectroscopy. No sign of the existence of the *trans* isomer was detected. Moreover, the crude hydrocarbon fraction was analyzed by vpc,[11] and no peak corresponding to the *trans* isomer was detected.

(1) R. M. Etter, H. S. Skovronek, and P. S. Skell, *J. Am. Chem. Soc.*, 81, 1008 (1959).
(2) (a) G. L. Closs and L. E. Closs, *Angew. Chem.*, 74, 431 (1962); (b) E. Funakubo, I. Moritani, T. Nagai, S. Nishida, and S. Murahashi, *Tetrahedron Letters*, 1069 (1963); (c) C. D. Gutsche, G. L. Backman, and R. S. Coffey, *Tetrahedron*, 18, 617 (1962); (d) P. S. Skell and J. Klebe, *J. Am. Chem. Soc.*, 82, 247 (1960).
(3) M. Jones, Jr., and K. R. Rettig, *ibid.*, 87, 4013, 4105 (1965).
(4) W. B. DeMore and S. B. Benson, *Advan. Photochem.*, 2, 219 (1964).
(5) P. P. Gaspard and G. S. Hammond, "Carbene Chemistry," W. Kirmse, Ed., Academic Press Inc., New York, N. Y., 1964, p 235.
(6) The addition of phenylcarbene to 2-butene is not completely stereospecific; the product from *cis*-2-butene was contaminated with 2.5% 3-phenyl-*trans*-1,2-dimethylcyclopropane.[2c]
(7) I. Moritani, S. Murahashi, M. Nishino, Y. Yamanoto, K. Itoh, and N. Mataga, *J. Am. Chem. Soc.*, 89, 1259 (1967).
(8) (a) W. Treibs and H.-J. Klinkhammer, *Chem. Ber.*, 84, 671 (1951); (b) T. W. Campbell, R. Ginsig, and H. Schmid, *Helv. Chim. Acta*, 36, 1489 (1953).
(9) W. Tochterman, K. Oppenländer, and U. Walter, *Chem. Ber.*, 97, 1329 (1964).
(10) Halos POH 1000 Eikosha Co. Ltd., Osaka, Japan.
(11) The crude hydrocarbon fraction was analyzed by vpc (Apieson L, 2 m, at 240°). No isomerization and decomposition was observed in this condition.